PCT/EP 03/07028
536747

Europäisches Patentamt
European Patent Office
Office européen des brevets

10/519210

REC'D 16 SEP 2003
WIPO PCT

Bescheinigung Certificate Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

02015067.8

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 02015067.8
Demande no:

Anmeldetag:
Date of filing: 05.07.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

BASF AKTIENGESELLSCHAFT

67056 Ludwigshafen
ALLEMAGNE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Plasmid vectors for transformation of filamentous fungi

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C12N15/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

Plasmid vectors for transformation of filamentous fungi

Description

The invention relates to novel plasmid vectors for transformation of filamentous fungi and to a method of modifiying the genome of filamentous fungi based on these vectors. The invention furthermore relates to the modification of a specific gene via the process of homologous recombination, to recombinant expression of foreign genes in filamentous fungi and to new markers for detecting the succesfull transfer of the target gene.

One method currently used for transformation of filamentous fungi is random mutagenesis based on transposons insertion, a method also known for plant transformation (WO 01/38509). This method allows to study the genoms for several species such as Magnaporthe grisea (for examples WO 00/55346; WO 00/56902). However, this strategy requires a big effort in term of bioinformatic tools and molecular biology to localise precisely the insertion in the genome.

Alternatively known transformation methods are based on targeted integration. Targeted transformation of fungi can be carried out either by offering a Knock out cassette with a marker-gene flanked by 2 homologous sequences (Aronson et al, 1994, Mol. Gen. Genet. 242: 490-494; Royer et al, 1999, Fungal Genetics and Biology 28: 68-78; Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150) or by quoting a plasmid with the marker gene in the neighbourship of a homologous sequence (Shortle et al.,1982, Science 217: 371-373; Bird and Bradshaw, 1997, Mol Gen Genet. 255: 219-225; Feng et al., 2001, Infection and Immunity 69 (3): 1781-1794; Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150). Both procedures are attractive methods to study the gene function, but they show also a high frequency of integration at ectopic sites by illegitimate recombination. The gene targeting efficiency (gene targeting / gene targeting + illegitimate recombination) is 95% for S. cerevisiae, 10-90% for S. pombe, 5-75% for Aspergillus nidulans and 1-30% for Neuropsora crassa using a size of homology of 2-9 Kb (Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150). Especially for filamentous fungi this side effect is quite high, if conventional plasmid vectors are used.

In addition, the efficiency of the gene targeting increases if the length of homologous recombination region is rised (Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150). Thus, plasmid

vectors currently used comprise gene fragments of the gene to be knocked out of a size of at least 2000bp as indicated above. The overall size of these plasmid vectors is at least 8000bp (P. J. Punt et. al., 1992, Methods in Enzymology, Vol.216, pp 447-457; ; Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150). Since transformation efficiency is decreasing by increase of the plasmid vector size, transformation efficiency is unsatisfactury giving rise to long times until positive clones can be identified. This is an obstacle especially for large scale genomic analysis projects or recombinant expression.

Furthermore, currently used plasmid vectors contain many unique restriction sites, rising difficulties in construction of the KO-plasmids and the transformation process. The efficiency of homologous recombination is improved when the KO-plasmid is digested with a restriction enzyme presenting a unique site in the middle of DNA fragment homologous to the targeted gene. The presence of high amount of restriction sites especially unique ones in the plasmid backbone decreaase the chance to find a natural restriction site in the appropriate location of the targeted DNA fragment. This problem is usually overcome by modification of the targeted DNA fragment requiring several cloning step and additional manipulation in terms of molecular biology, what is a disadvantageous time consuming methology.

Integration of recombinant gene by homolgous recombination in fungi is a also a tool to identify gene function for essential gene: the biochemical characterization of an essential gene cannot be studied by classical knock-out strategy since the mutants carrying a disruption of such a gene are not viable. One way consisting to overexpress such a gene overcome the problem when a typical phenotype can be assigned to the mutant that overexpresses the gene. Another approach can be to regulate the gene expression by an inducible promoter sequence so that the gene could be expressed or repressed when needed and consequently permits to isolate viable mutants. As mentioned above, these approaches require at least several thousand bp of the nucleic acid sequence to be studied that need to be integrated in the genome of the fungi together with a plasmid vector comprising the different parts of the nucleic acid sequence. In addition, if the recombinant DNA is integrated at an ectopic site, the identification of the mutant strains becomes more complicated and the position of the integration in the genome may influence the level of expression of the recombinant protein. Taking the aforesaid into consideration, currently existing plasmid vectors currently used for transformation of filamentous fungi exhibit a lot of disadvantages and are neither suitable for large scale analysis e.g. in

functional genomic studies nor convenient for recombinant expression in a filamentous fungi. Additionally, there is a constant need for new selection markers facilitating the selection process.

Thus, object of the present invention was to develop tools for targeted transformation of filamentous fungi that overcome the disadvantages of the state of the art like plasmid vectors suitable for functional genomic studies and recombinant expression and new selection markers.

Surprisingly, we have found that the object of the invention has been achieved by construction of a plasmid vector for targeted transformation of filamentous fungi comprising

a) an origin of replication for a host organism which is not originating from the filamentous fungi to be transformed;

b) a selection marker for a host organism not originating from the filamentous fungi to be transformed;

c) a promotor facilitating recombinant expression in filamentous fungi that is functionally linked to the coding region of the hygromycin resistance gene which is functionally linked to a terminator which facilitates transcription termination in filamentous fungi;

wherein the overall size of the elements a), b) and c) does not exceed 4500 bp; and

d) a nucleic acid sequence, which is homologous to nucleic acid sequences of the filamentous fungi to be transformed and makes homologous recombination in the filamentous fungi to be transformed possible.

The overall size of the elements a), b) and c) does not exceed 4500 bp, preferaby 4100 bp, more preferably 3700 bp.

In addition to the nucleic acid elements a), b), c) and d) the plasmid vector optionally comprises a cloning site containing rare restriction sites or a TA-cloning site by which further nucleic acid sequences can been cloned easily into the plasmid vector. A TA-cloning site comprises thymidine residues linked onto the 3'-ends of linearized plasmid DNA, which would allow some annealing to occur between the vector and the A-tailed PCR product to be ligated. This process is called TA cloning. Preferably, the vector is modified that there are only few unique restriction

sites left enabling the digestion by commercial available restriction enzymes of the homologous sequence of the targeted gene prior to the transformation.

Filamentous fungi that can be transformed with the vectors of the present invention are non-phytopathogenic filamentous fungi e.g. Neurospora species like Neurospora crassa and phytopathogenic filamentous fungi, wherein the phytopathogenic filamentous fungi are preferred. Preferred phytopathogenic filamentous fungi are selected from the group consiting of the genera Neurospora, Alternaria, Podosphaera, Sclerotinia, Physalospora, Botrytis, Corynespora; Colletotrichum; Diplocarpon; Elsinoe; Diaporthe; Sphaerotheca; Cinula, Cercospora; Erysiphe; Sphaerotheca; Leveillula; Mycosphaerella; Phyllactinia; Gloesporium; Gymnosporangium, Leptotthrydium; Podosphaera; Gloedes; Cladosporium; Phomopsis; Phytopora; Phytophthora; Erysiphe; Fusarium; Verticillium; Glomerella; Drechslera; Bipolaris; Personospora; Phaeoisariopsis; Spaceloma; Pseudocercosporella; Pseudoperonospora; Puccinia; Typhula; Pyricularia; Rhizoctonia; Stachosporium; Uncinula; Ustilago; Gaeumannomyces and Fusarium, more preferred from the group consisting of the genera and species Neurospora such as Neurospora crassa, Alternaria, Podosphaera, Sclerotinia, Physalospora such as Physalospora canker, Botrytis species such as Botrytis cinerea, Corynespora such as Corynespora melonis; Colletotrichum; Diplocarpon such as Diplocarpon rosae; Elsinoe such as Elsinoe fawcetti, Diaporthe such as Diaporthe citri; Sphaerotheca; Cinula such as Cinula neccata, Cercospora; Erysiphe such as Erysiphe cichoracearum and Erysiphe graminis; Sphaerotheca such as Sphaerotheca fuliginea; Leveillula such as Leveillula taurica; Mycosphaerella; Phyllactinia such as Phyllactinia kakicola; Gloesporium such as Gloesporium kaki; Gymnosporangium such as Gymnosporangium yamadae, Leptotthrydium such as Leptotthrydium pomi, Podosphaera such as Podosphaera leucotricha; Gloedes such as Gloedes pomigena; Cladosporium such as Cladosporium carpophilum; Phomopsis; Phytopora; Phytophthora such as Phytophthora infestans; Verticillium; Glomerella such as Glomerella cingulata; Drechslera; Bipolaris; Personospora; Phaeoisariopsis such as Phaeoisariopsis vitis; Spaceloma such as Spaceloma ampelina; Pseudocercosporella such as Pseudocercosporella herpotrichoides; Pseudoperonospora; Puccinia; Typhula; Pyricularia such as Pyricularia oryzae; Rhizoctonia; Stachosporium such as Stachosporium nodorum; Uncinula such as Uncinula necator; Ustilago; Gaeumannomyces species such as Gaeumannomyces graminis and Fusarium such as Fusarium dimerium, Fusarium merismoides, Fusarium lateritium, Fusarium decemcellulare, Fusarium poae, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium chlamydosporum, Fusarium moniliforme, Fusarium proliferatum, Fusarium anthophilum, Fusarium subglutinans, Fusa-

rium nygamai, Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium sambucinum, Fusarium crookwellense, Fusarium avenaceum ssp. avenaceum, Fusarium avenaceum ssp. aywerte, Fusarium avenaceum ssp. nurragi, Fusarium hetrosporum, Fusarium acuminatum ssp. acuminatum, Fusarium acuminatum ssp. armeniacum, Fusarium longipes, Fusarium compactum, Fusarium equiseti, Fusarium scripi, Fusarium polyphialidicum, Fusarium semitectum and Fusarium beomiforme and especially preferred from the genera Fusarium such as Fusarium graminearum, most preferred from the group consisting of the genera and species Fusarium, Fusarium dimerium, Fusarium merismoides, Fusarium lateritium, Fusarium decemcellulare, Fusarium poae, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium chlamydosporum, Fusarium moniliforme, Fusarium proliferatum, Fusarium anthophilum, Fusarium subglutinans, Fusarium nygamai, Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium sambucinum, Fusarium crookwellense, Fusarium avenaceum ssp. avenaceum, Fusarium avenaceum ssp. aywerte, Fusarium avenaceum ssp. nurragi, Fusarium hetrosporum, Fusarium acuminatum ssp. acuminatum, Fusarium acuminatum ssp. armeniacum, Fusarium longipes, Fusarium compactum, Fusarium equiseti, Fusarium scripi, Fusarium polyphialidicum, Fusarium semitectum and Fusarium beomiforme wherein Fusarium graminearum is most preferred.

The host organism in which the origin of replication a) is functionally active essentially serves for constructing and propagating the plasmid vector of the invention. The host organism must be genetically different from the filamentous fungi to be transformed, since replication of the plasmid vector should not take place in the filamentous fungi to be transformed but is desired in the host organism, due to using the origin of replication a). Host organisms which may be used are all common microorganisms which can easily be manipulated by genetic engineering. Preferred host organisms are Gram-negative bacteria such as the genera Escherichia and Salmonella e.g. Escherichia coli and Salmonella thyplimurium or Gram-positive bacteria such as the genera Bacillus and Streptomyces, e.g. Bacillus subtilis and Streptomyces nidulans. Particularly preferred are Gram-negative bacteria such as Escherichia, e.g. Escherichia coli.

Preferred origins of replications (ori) are the col E1 ori, the f1 ori.

The selection marker b) means a gene or the expression product of the gene. Preferred meanings are genes whose expression causes resistance of the host organism to antibiotics, by preference a resistance to kanamycin, chloramphenicol, tetracycline, zeocin or

ampicillin and particualry preferred ampicillin and kanamycin.

In a preferred embodiment, the element a) of the plasmid vector according to the invention comprises an col E1 origin of replication and the ampicillin resistance gene as selection marker for the host organism.

The element c) is hereinbelow termed as "hygromycin cassette". The coding region of the hygromycin resistance gene (hereinbelow termed "hygromycin gene") is known by the skilled artisan (Gritz L. and Davies J. ,1983, Gene 25, 179-188, Kaster, K.R., Burgett S.G. and Ingolia T.D., 1984, Curr. Genet. 8,353-358) and has a length of 1026bp.

Examples of suitable promotors to which the coding region of the hygromycin gene is functionally linked to, are the GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5-, AOX1, GAL10/CYC-1, CYC1, OliC-, ADH-, TDH-, Kex2-, MFα- or the NMT-promotor (Degryse et al., Yeast 1995 Jun 15; 11(7):629-40; Romanos et al. Yeast 1992 Jun;8(6):423-88; Benito et al. Eur. J. Plant Pathol. 104, 207-220 (1998); Cregg et al. Biotechnology (N Y) 1993 Aug;11(8):905-10; Luo X., Gene 1995 Sep 22;163(1):127-31; Nacken et al., Gene 1996 Oct 10;175(1-2):253-60; Turgeon et al., Mol Cell Biol 1987 Sep;7(9):3297-305), preferably the CYC1-, ADH-, TDH-, Kex2-, GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5- or AOX1-promotor, more preferably the GPD-1-, PX6, TEF- or the CUP1-promotor, most preferably the GPD1 or the TEF-promotor.

Examples of suitable terminators that are functionally linked to the coding region of the hygromycin gene are the AOX1-, nos-, PGK-, TrpC- or the CYC1-terminator (Degryse et al., Yeast 1995 Jun 15; 11(7):629-40; Brunelli et al. Yeast 1993 Dec9(12): 1309-18; Frisch et al., Plant Mol. Biol. 27 (2), 405-409 (1995); Scorer et al., Biotechnology (N.Y.) 12 (2), 181-184 (1994), Genbank acc. number Z46232; Punt et al., (1987) Gene 56 (1), 117-124)); preferably the CYC1- or nos-terminator, more preferably the nos-terminator.

In a preferred embodiment, the hygromycin cassette comprises a GPD-1 promotor functionally linked to the coding region of the hygromycin region which is functionally linked to the nos-terminator.

A functional linkage is understood as meaning the sequential arrangement of promoter and coding sequence, of coding sequence and terminator or of promoter, coding sequence and terminator in such a way that each of the regulatory elements can, upon expression

of the coding sequence, fulfil its function upon the recombinant expression of the nucleic acid sequence. Direct linkage in the chemical sense is not necessarily required for this purpose. Preferred arrangements are those in which the hygromycin gene to be expressed recombinantly is positioned downstream of the sequence which acts as promoter, so that the two sequences are linked covalently to each other. The distance between the promoter sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 100 base pairs, especially preferably less than 50 base pairs, very especially preferably less than 10 base pairs. The distance between the terminator sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 100 base pairs, especially preferably less than 50 base pairs, very especially preferably less than 10 base pairs. However, further sequences which, for example, exert the function of a linker with certain restriction enzyme cleavage sites, or of a signal peptide, may also be positioned between the two sequences.

These vectors are not only much more smaller than currently used plasmid vectors, but exhibit also a high transformation efficiency. Surprisingly, a high transformation efficiency can be gained even if small DNA-fragments of at least 300bp, preferably at least 400bp, more preferably at least 450bp, most preferably at least 500bp of the nucleic acid sequence d) to be analyzed are used. The average degree of illegitimate recombination is below 30%, preferably between 0 to 20%.

The nucleic acid sequence d) has a homology of at least 80% to the nucleic acid sequence of the filamentous fungi to be transformed, preferably at least 90%, more preferably at least 95% and most preferably at least 100%.

In a preferred embodiment, the nucleic acid sequence d) originates from a filamentous fungi and has a length of at least 400bp, more preferably at least 450bp, most preferably at least 500bp. These length are suitable for functional genomic studies. Also nucleic acid sequences exceeding 500bp can be used, e.g. for the purpose recombinant expression.

If the nucleic acid sequence d) is to be expressed recombinantly in the filamentous fungi, it can be functionally linked to a promotor e) and optionally to a terminator f).

Examples of suitable promotors e) are for example the AUG1-, GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5-, AOX1, GAL10/CYC-1, CYC1, O1iC-, ADH-, TDH-, Kex2-, MFα- or the NMT-pro-

motor or combinations of the aforementioned promotors (Degryse et al., Yeast 1995 Jun 15; 11(7):629-40; Romanos et al. Yeast 1992 Jun;8(6):423-88; Benito et al. Eur. J. Plant Pathol. 104, 207-220 (1998); Cregg et al. Biotechnology (N Y) 1993 Aug;11(8):905-10; Luo X., Gene 1995 Sep 22;163(1):127-31; Nacken et al., Gene 1996 Oct 10;175(1-2): 253-60; Turgeon et al., Mol Cell Biol 1987 Sep;7(9):3297-305).

Examples of suitable terminators f) are the NMT-, Gcy1-, TrpC-, AOX1-, nos-, the PGK- or the CYC1-terminator (Degryse et al., Yeast 1995 Jun 15; 11(7):629-40; Brunelli et al. Yeast 1993 Dec9(12): 1309-18; Frisch et al., Plant Mol. Biol. 27 (2), 405-409 (1995); Scorer et al., Biotechnology (N.Y.) 12 (2), 181-184 (1994), Genbank acc. number Z46232; Zhao et al. Genbank acc number : AF049064; Punt et al., (1987) Gene 56 (1), 117-124).

The nucleic acid sequence d) can be also functionally linked to an affinity tag to purify the encoded protein and/or to a reporter gene to study biochemical properties of the nucleic acid sequence d) in vivo, respectively.

"Reporter genes" encode readily quantifiable proteins. Using these genes, an assessment of transformation efficacy or of the site or time of expression can be made via growth, fluorescence, chemoluminescence, bioluminescence or resistance assay or via photometric measurement (intrinsic color) or enzyme activity. Very especially preferred in this context are reporter proteins (Schenborn E, Groskreutz D. Mol. Biotechnol. 1999; 13(1):29-44) such as the "green fluorescence protein" (GFP) (Gerdes HH and Kaether C, FEBS Lett. 1996; 389(1):44-47; Chui WL et al., Curr. Biol. 1996, 6:325-330; Leffel SM et al., Biotechniques. 23(5):912-8, 1997), chloramphenicol acetyl transferase, a luciferase (Giacomin, Plant Sci. 1996, 116:59-72; Scikantha, J. Bact. 1996, 178:121; Millar et al., Plant Mol. Biol. Rep. 1992 10:324-414), and luciferase genes, in general β-galactosidase or β-glucuronidase (Jefferson et al., EMBO J. 1987, 6, 3901-3907), the Ura3 gene, the Ilv2 gene, the 2-desoxyglucose-6-phosphate phosphatase gene, β-lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase gene, or the BASTA (= gluphosinate) resistance gene.

The term "affinity tag" denotes a peptide or polypeptide whose coding nucleic acid sequence can be fused to the nucleic acid sequence d) either directly or using a linker, by customary cloning techniques. The affinity tag serves to isolate the recombinant target protein by means of affinity chromatography. The abovemen-

tioned linker can optionally comprise a protease cleavage site (for example for thrombin or factor Xa), whereby the affinity tag can be cleaved off from the target protein, as required. Examples of customary affinity tags are the "his-tag", for example from Quiagen, Hilden, "strep-tag", "myc-tag" (Invitrogen, Carlsberg), New England Biolab's tag which consists of a chitin binding domain and an intein, and what is known as the CBD-tag from Novagen.

In a particularly preferred embodiment, the plasmid vector comprises an coli E1 ori, the ampicillin resistance gene as selection marker, a GPD-1 promotor functionally linked to the coding region of the hygromycin resistance gene which is functionally linked to the nos-terminator.

Preferably, the vector also comprises a multiple cloning site comprising appropriate restriction enzyme site. Appropriate restriction sites are well known by the skilled artisan.

In a further preferred embodiment, the plasmid vector additionaly comprises a TA-cloning site to facilitate the overall cloning procedure.

Examples of particularly preferred embodiments are set forth in Fig. 1 and 2.

All of the above-mentioned embodiments of plasmid vectors are hereinbelow termed as "plasmid vector (or vector) according to the invention".

If a plasmid is used for recombinant expression in a host, a marker is required indicating the sucessfull transfer of the plasmid vector DNA into the filamentous fungi to be transformed.

Surprisingly, we have found that the gene fragments of the polyketide synthase are a well suited selection marker. The term selection marker referred to the polyketide synthase herein means a nucleic acid sequence.

Polyketide synthases are multifunctional enzymes that are involved in the biosynthesis of several important polyketides. Polyketides constitute a large and highly diverse group of secondary metabolites, synthesized by bacteria, fungi and plants. They include antibiotics, compounds with mycotoxic activity, and compounds within pigment biosynthetic pathways. Further a polyketide synthase is described to be required for fungal virulence of Cochliobolus heterostrophous toward maize (Yang et al., 1996

PMID:8953776). Polyketide Synthetases are furthermore known from Wangiella dermatidis (pubMedID:11179356), from Aspergillus nidulans (Swiss-prot ID: Q03149; identity with SEQ ID No:4= 38%; identity with SEQ ID No:6= 40%; identity with SEQ ID No:8= 48%; identity with SEQ ID No:10= 57%; identity with SEQ ID No:12= 38,5%; identity with SEQ ID No:14= 46%) and from Aspergillus parasiticus (Swiss-Prot ID:Q12053; identity with SEQ ID No:4= 38%; identity with SEQ ID No:6= 38%; identity with SEQ ID No:8= 39%; identity with SEQ ID No:10= 48%; identity with SEQ ID No:12= 36%; identity with SEQ ID No:14= 32%).

The use of polyketide synthase as selectable marker to be used in an expression for filamentous fungi has not yet been described.

Thus, the present invention encompasses a selection marker comprising a nucleic acid sequence encoding a polyketide snythetase fragment, wherein said nucleic acid sequence comprises

i. a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2; or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14 by back translation; or

iii. functional equivalents of the nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by back translation from a functional equivalent of an amino acid sequence shown in SEQ ID NO:4 that has at least an identity of 39% with the SEQ ID NO:4 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 41% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 49% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of 58% with the SEQ ID NO:10 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:12 that has at least an identity of 39% with the SEQ ID NO:12 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:14 that has at least an identity of 46% with the SEQ ID NO:14; or

iv. functional equivalents of the nucleic acid sequence shown in SEQ ID NO:1 having at least an identity of 80% with the SEQ ID NO:1 or functional equivalents of SEQ ID NO:2 having at least an identity of 80% with the SEQ ID NO:2; or

v. parts of the nucleic acid sequence as defined in i), ii), iii) or iv) consisting of at least 300bp.

Parts or segments of nucleic acid sequences set forth in v) consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp, most preferably at least 500bp of the nucleic acid sequences. In a further preferred embodiment, those parts are selected from SEQ ID NO:1, preferably from 732bp to 5881bp of SEQ ID NO:1 e.g. from 2236bp to 2870bp.

Preferred are nucleic acid sequences as defined above originate from filamentous fungi, preferably phytopathogenic filamentous fungi selected from the group consiting of the genera Neurospora, Alternaria, Podosphaera, Sclerotinia, Physalospora, Botrytis, Corynespora, Colletotrichum; Diplocarpon; Elsinoe; Diaporthe; Sphaerotheca; Cinula, Cercospora; Erysiphe; Sphaerotheca; Leveillula; Mycosphaerella; Phyllactinia; Gloesporium; Gymnosporangium, Leptotthrydium, Podosphaera; Gloedes; Cladosporium; Phomopsis; Phytopora; Phytophthora; Erysiphe; Fusarium; Verticillium; Glomerella; Drechslera; Bipolaris; Personospora; Phaeoisariopsis; Spaceloma; Pseudocercosporella; Pseudoperonospora; Puccinia; Typhula; Pyricularia; Rhizoctonia; Stachosporium; Uncinula; Ustilago; Gaeumannomyces and Fusarium, more preferred from the group consisting of the genera and species Neurospora such as Neurospora crassa, Alternaria, Podosphaera, Sclerotinia, Physalospora such as Physalospora canker, Botrytis species such as Botrytis cinerea, Corynespora such as Corynespora melonis; Colletotrichum; Diplocarpon such as Diplocarpon rosae; Elsinoe such as Elsinoe fawcetti; Diaporthe such as Diaporthe citri; Sphaerotheca; Cinula such as Cinula neccata, Cercospora; Erysiphe such as Erysiphe cichoracearum and Erysiphe graminis; Sphaerotheca such as Sphaerotheca fuliginea; Leveillula such as Leveillula taurica; Mycosphaerella; Phyllactinia such as Phyllactinia kakicola; Gloesporium such as Gloesporium kaki; Gymnosporangium such as Gymnosporangium yamadae, Leptotthrydium such as Leptotthrydium pomi, Podosphaera such as Podosphaera leucotricha; Gloedes such as Gloedes pomigena; Cladosporium such as Cladosporium carpophilum; Phomopsis; Phytopora; Phytophthora such as Phytophthora infestans; Verticillium; Glomerella such as Glomerella cingulata; Drechslera; Bipolaris; Personospora; Phaeoisariopsis such as Phaeoisariopsis vitis; Spaceloma such as Spaceloma ampelina; Pseudocercosporella such as Pseudocercosporella herpotrichoides; Pseudoperonospora;

Puccinia; Typhula; Pyricularia such as Pyricularia oryzae; Rhizoctonia; Stachosporium such as Stachosporium nodorum; Uncinula such as Uncinula necator; Ustilago; Gaeumannomyces species such as Gaeumannomyces graminis and Fusarium such as Fusarium dimerium, Fusarium merismoides, Fusarium lateritium, Fusarium decemcellulare, Fusarium poae, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium chlamydosporum, Fusarium moniliforme, Fusarium proliferatum, Fusarium anthophilum, Fusarium subglutinans, Fusarium nygamai, Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium sambucinum, Fusarium crookwellense, Fusarium avenaceum ssp. avenaceum, Fusarium avenaceum ssp. aywerte, Fusarium avenaceum ssp. nurragi, Fusarium hetrosporum, Fusarium acuminatum ssp. acuminatum, Fusarium acuminatum ssp. armeniacum, Fusarium longipes, Fusarium compactum, Fusarium equiseti, Fusarium scripi, Fusarium polyphialidicum, Fusarium semitectum and Fusarium beomiforme and especially preferred from the genera Fusarium such as Fusarium graminearum, most preferred from the group consisting of the genera and species Fusarium, Fusarium dimerium, Fusarium merismoides, Fusarium lateritium, Fusarium decemcellulare, Fusarium poae, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium chlamydosporum, Fusarium moniliforme, Fusarium proliferatum, Fusarium anthophilum, Fusarium subglutinans, Fusarium nygamai, Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium sambucinum, Fusarium crookwellense, Fusarium avenaceum ssp. avenaceum, Fusarium avenaceum ssp. aywerte, Fusarium avenaceum ssp. nurragi, Fusarium hetrosporum, Fusarium acuminatum ssp. acuminatum, Fusarium acuminatum ssp. armeniacum, Fusarium longipes, Fusarium compactum, Fusarium equiseti, Fusarium scripi, Fusarium polyphialidicum, Fusarium semitectum and Fusarium beomiforme wherein Fusarium graminearum is most preferred.

The term "comprising" means that the nucleic acid sequence according to the invention can be flanked by additional nucleic acid sequences that have on the 5' end a sequence length of at least 100 bp and preferably at least 50 bp, more preferably at least 20bp, most preferably at least 10bp and on the 3' a sequence length of at least 4000 bp and preferably at least 3000 bp, more preferably at least 2500 bp.

"Functional equivalents" in the present context describe nucleic acid sequences which hybridize under standard conditions with the nucleic acid sequence or portions of the nucleic acid sequence having the function of the a selection marker.

It is advantageous to use short oligonucleotides of a length of 10-50 bp, preferably 15-40 bp, for example of the conserved or other regions, which can be determined via comparisons with other

related genes in a manner known to the skilled worker for the hybridization. Alternatively, it is also possible to use longer fragments of the nucleic acids according to the invention or the complete sequences for the hybridization. These standard conditions vary depending on the nucleic acid used, viz. oligonucleotide, longer fragment or complete sequence, or depending on which type of nucleic acid, viz. DNA or RNA, is being used for the hybridization. Thus, for example, the melting temperatures for DNA:DNA hybrids are approx. 10°C lower than those of DNA:RNA hybrids of equal length.

Standard conditions are understood as meaning, depending on the nucleic acid, for example temperatures between 42 and 58°C in an aqueous buffer solution with a concentration of between 0.1 and 5 x SSC (1 x SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.2) or additionally in the presence of 50% formamide such as, for example, 42°C in 5 x SSC, 50% formamide. The hybridization conditions for DNA:DNA hybrids are advantageously 0.1 x SSC and temperatures of between approximately 20°C and 45°C, preferably between approximately 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are advantageously 0.1 x SSC and temperatures of between approximately 30°C and 55°C, preferably between approximately 45°C and 55°C. These temperatures stated for the hybridization are melting temperature values which have been calculated by way of example for a nucleic acid with a length of approx. 100 nucleotides and a G + C content of 50% in the absence of formamide. The experimental conditions for DNA hybridization are described in specialist textbooks of genetics such as, for example, Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989 and can be calculated using formulae known to the skilled worker, for example as a function of the length of the nucleic acids, the type of the hybrids or the G + C content. The skilled worker can find more information on hybridization in the following textbooks: Ausubel et al. (eds), 1985, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Hames and Higgins (eds), 1985, Nucleic Acids Hybridization: A Practical Approach, IRL Press at Oxford University Press, Oxford; Brown (ed), 1991, Essential Molecular Biology: A Practical Approach, IRL Press at Oxford University Press, Oxford.

A functional equivalent is furthermore also understood as meaning, in particular, natural or artificial mutations of the relevant nucleic acid sequences of the PKS and its homologs from other organisms, wherein mutations comprise substitutions, additions, deletions, inversions or insertions of one or more nucleotide residues. This may also lead to a modification of the corresponding amino acid sequence of the PKS by substitution, inser-

tion or deletion of one or more amino acids.

Thus, the scope of the present invention also extends to, for example, those nucleotide sequences which are obtained by modification of the nucleic acid sequence of the selection marker described by SEQ ID NO:1 or by SEQ ID NO:2. The purpose of such a modification can be, for example, the insertion of further cleavage sites for restriction enzymes, the removal of excess DNA, or the addition of further sequences. Proteins which are encoded via said nucleic acid sequences should still maintain the desired functions, despite the deviating nucleic acid sequence.

The functional equivalents of SEQ ID NO:1 set forth in iv) which are according to the invention and claimed herein have preferably at least 80%, by preference at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, especially preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID No:1. Said functional equivalents are distinguished by an essentially identical functionality that means they are functional selection markers for filamentous fungi.

The functional equivalents of SEQ ID NO:2 set forth in iv) which are according to the invention and claimed herein have preferably at least 80%, by preference at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, especially preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID No:2. Said functional equivalents are distinguished by an essentially identical functionality that means they are functional selection markers for filamentous fungi.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:4 by back translation having at least an identity of 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60% preferred of 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% more preferred of 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID No:4. Said functional equivalents are distinguished by an essentially identical functionality with SEQ ID NO:4 by an essentially identical functionality that means they are functional selection markers for filamentous fungi.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation having at least an identity of 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60% preferred of 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% more preferred of 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID No:6. Said functional equivalents are distinguished by an essentially identical functionality with SEQ ID NO:6 by an essentially identical functionality that means they are functional selection markers for filamentous fungi.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:8 by back translation having at least an identity of 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70% preferred of 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% more preferred of 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID No:8. Said functional equivalents are distinguished by an essentially identical functionality with SEQ ID NO:8 by an essentially identical functionality that means they are functional selection markers for filamentous fungi.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10 by back translation having at least an identity of 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70% preferred of 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% more preferred of 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID No:10. Said functional equivalents are distinguished by an essentially identical functionality with SEQ ID NO:10 by an essentially identical functionality that means they are functional selection markers for filamentous fungi.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:12 by back translation having at least an identity of 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48% 49%, 50%, 51%, 52%, 53%, 54%,

55%, 56%, 57%, 58%, 59%, 60% preferred of 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% more preferred of 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID No:12. Said functional equivalents are distinguished by an essentially identical functionality with SEQ ID NO:12 by an essentially identical functionality that means they are functional selection markers for filamentous fungi.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:14 by back translation having at least an identity of 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70% preferred of 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% more preferred of 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID No:14. Said functional equivalents are distinguished by an essentially identical functionality with SEQ ID NO:14 by an essentially identical functionality that means they are functional selection markers for filamentous fungi.

The term "identity" or "homology" between two nucleic acid sequences or polypeptide sequences is defined by the identity of the nucleic acid sequence/polypeptide sequence by in each case the entire sequence length, which is calculated by alignment with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap Weight: 8 Length Weight: 4

Average Match: 2,912 Average Mismatch:-2,003

The term homology if used herein is the same as the term identity.

Functional equivalents thus encompass naturally occurring variants of the sequences described herein, and also artificial, for example chemically synthesized, nucleic acid sequences adapted to the codon usage, or the amino acid sequences derived therefrom.

The invention furthermore relates to the use of SEQ ID NO:PKS, of functional equivalents and of segments SEQ ID NO:PKS as marker for targeted transformation in filamentous fungi.

Further encompassed by the present invention is the use of a nucleic acid sequence as marker for targeted transformation in filamentous fungi said nucleic acid comprising

i. a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2; or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14 by back translation; or

iii. functional equivalents of the nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by back translation from a functional equivalent of an amino acid sequence shown in SEQ ID NO:4 that has at least an identity of 37% with the SEQ ID NO:4 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 38% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 39% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of 48% with the SEQ ID NO:10 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:12 that has at least an identity of 36% with the SEQ ID NO:12 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:14 that has at least an identity of 32% with the SEQ ID NO:14; or

iv. functional equivalents of the nucleic acid sequence shown in SEQ ID NO:1 having at least an identity of 80% with the SEQ ID NO:1 or functional equivalents of SEQ ID NO:2 having at least an identity of 80% with the SEQ ID NO:2; or

v. parts of the nucleic acid sequence as defined in i), ii), iii) or iv) consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp, most preferably at least 500bp

Preferred filamentous fungi are those mentioned above. The aforementioned nucleic acid sequences are hereinbelow also termed "PKS-marker".

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO: 4 by back translation having at least an identity of 37%, 38% preferred of 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65% more preferred of 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% with the SEQ ID NO:4.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation having at least an identity of 38%, 39%, 40% preferred of 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65% more preferred of 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% with the SEQ ID NO:6.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO: 8 by back translation having at least an identity of 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, preferred of 49%, 50%, 51% 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70% more preferred of 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% with the SEQ ID NO:8.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO: 10 by back translation having at least an identity of 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, preferred of 58%, 59% 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75% more preferred of 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% with the SEQ ID NO:10.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO: 12 by back translation having at least an identity of 36%, 37%, 38% preferred of 39%, 40% 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60% more preferred 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85% most preferred of 86%, 88%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% with the SEQ ID NO:12.

The functional equivalents of the nucleic acid sequence set forth in iii) which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:14 by back translation having at least an identity of 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45% preferred of 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59% 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70% more preferred of 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 88%, 88%, 89%, 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% with the SEQ ID NO:14.

Another embodiment of the present invention are plasmid vectors for targeted transformation of filamentous fungi comprising a PKS-marker. These plasmid vectors are either vectors currently used for targeted transformation of filamentous fungi e.g., such as pAN7 (Punt et al, 1987 Gene 36:117-124) and other vectors that are well known by the skilled artisan or plasmid vectors according to the invention, preferably plasmid vectors according to the invention.

All of the above-mentioned vectors comprising the PKS gene-fragment are hereinbelow termed as "PKS-vectors".

All vectors according to the invention not comprising the PKS gene-fragment as set forth in i) to iv) are hereinbelow termed as "non-PKS-vectors".

The present invention furthermore encompasses a method for preparing mutated filamentous fungi, comprising the steps of transferring a non-PKS vector or a PKS-vector into a filamentous fungi; and selecting clones of said filamentous fungi, which contain at least one genetic marker introduced by said plasmid vector.

The term filamentous fungi as well as preferred fungi for this purpose are those mentioned and defined above.

In a preferred embodiment, the method for preparing mutated filamentous fungi, comprising the following steps

a) transferring a PKS-vector into a filamentous fungi; and

b) selecting successfully transformed filamentous fungi by the absence of colour.

If a PKS-vector is transferred into a filamentous fungi, the disruption of the PKS gene leads to a loss of colour whereby the degree of transformation can be determined easily. Resulting transformants are white in contrast to the colored wild-type. Thus, the selection according to step b) is done by monitoring the absence of melanin in the filamentous fungi. In a preferred embodiment, the absence of pigment is monitored by optical means.

In a more preferred embodiment, the PKS-vector comprises at least an additional selection marker, preferably the hygromycin resistance gene. In a particular preferred embodiment, the selection of the succesfully transformed filamentous fungi comprising a PKS-vector can be carried out by hygromycin resistance of sucessfully transformed clones and by the absence of pigment of sucessfully transformed clones. Most preferably, the PKS-vector is a vector according to the invention additionally comprising a PKS-marker.

In a further embodiment of the invention, the selection of the succesfully transformed filamentous fungi comprising a non-PKS-vector can be carried out by hygromycin resistance of sucessfully transformed clones.

If a non-PKS-vector is used, the vector is linearized by a restriction enzyme cutting in the nucleic acid sequence region of element d). Also nucleic acid sequences exceeding 2000 bp can be used what can be disadvantageous as mentioned above: If a PKS-vector is used, the plasmid vector is transferred into a filamentous fungi with the proviso said vector beeing linerazed by a restriction enzyme in PKS-marker nucleic acid sequence. Contrarily to the non-PKS-vectors, the nucleic acid sequence to be expressed recombinantly can also be smaller than 400bp.

In addition to the aforementioned selection methods set forth in step a) to c), homologous recombination can be confirmed by PCR based on oligonucleotides preferably derived from the vector se-

quence flanking the 5 and 3 region of the gene to be inserted. Specific examples of these primers are given in the examples.

The plasmid vector may be transferred into the filamentous fungi to be transformed by methods familiar to the skilled worker, preferably via protoplast preparation with driselase or driselase and glucanase as lytic enzyme.

The above-mentioned transformation methods can be also realized in a high throughput screening. Using high throughput screening, many different clones are obtained in parallel so that large numbers of sucessfully tranformed clones of filamentous fungi can be quickly screened.

The term filamentous fungi as well as preferred fungi for this purpose are those mentioned and defined above.

Due to the convenience of the vector, the above-mentioned KO-plasmid preparation, fungi transformation and screening of the mutants can be at least partially automated so that the whole procedure can also be realized in a high throughput screening. Using high throughput system for example for KO-plasmid preparation and DNA amplification by PCR to screen the recombinant mutants, many different clones are obtained in parallel so that large numbers of sucessfully tranformed clones can be quickly screened.

Mutagenized filamentous fungi, obtainable according to a method mentioned above, are further encompassed by the present invention.

The invention is now illustrated by the examples which follow, but not limited thereto.

Examples

The recombinant methods on which the exemplary embodiments which follow are based are now described briefly:

A: General methods

Cloning methods such as, for example, restriction cleavages, DNA isolation, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking of DNA fragments, transformation of E. coli cells, bacterial cultures, sequence analysis of recombinant DNA

and Southern and Western Blots were carried out as described by Sambrook et al., Cold Spring Harbor Laboratory Press (1989) and Ausubel, F.M. et al., Current Protocols in Molecular Biology. Greene Publishing Assoc. and Wiley-Interscience (1994); ISBN 0-87969-309-6.

The bacterial strains used hereinbelow (E. coli DH5 or XL1 blue) were obtained from Life Technologies or Stratagene . The vector were used for cloning. DSM:4527 can be used as F. Graminearum wild-type strain 8/1 verwendet werden. Restriction maps of the vectors pUCmini-Hyg and PUCmini-Hyg TA are given in Fig 1 and 2.

B: Sequence analysis of recombinant DNA (please check, wether this is the method of choice)

Recombinant DNA molecules were sequenced using an ABI laser fluorescence DNA sequencer following the method of Sanger (Sanger et al., Proc. Natl. Acad. Sci. USA, 74, 5463-5467(1977)). Fragments resulting from a polymerase chain reaction were sequenced and verified in order to avoid polymerase errors in constructs to be expressed.

C: Materials used

Unless otherwise specified in the text all of the chemicals used were obtained in analytical grade quality from Fluka (Neu-Ulm), Merck (Darmstadt), Roth (Karlsruhe), Serva (Heidelberg) and Sigma (Deisenhofen). Solutions were prepared using pure pyrogen-free water, referred to in the following text as H_2O, from a Milli-Q water system purification unit (Millipore, Eschborn). Restriction enzymes, DNA-modifying enzymes and molecular-biological kits were obtained from AGS (Heidelberg), Amersham (Brunswick), Biometra (Göttingen), Roche (Mannheim), Genomed (Bad Oeynnhausen), New England Biolabs (Schwalbach/Taunus), Novagen (Madison, Wisconsin, USA), Perkin-Elmer (Weiterstadt), Pharmacia (Freiburg), Qiagen (Hilden) and Stratagene (Heidelberg). Unless otherwise specified, they were used following the manufacturer's instructions.

All of the media and buffers used for the genetic engineering experiments were sterilized either by filter sterilization or by heating in the autoclave.

Examples

Example 1 - Construction of pUCmini-Hyg and PUCmini-Hyg TA vector

A 2536 bp DNA fragment corresponding to the promoter of glycerol-3-phosphate dehydrogenase (GPD1) from *Cochliobolus heterotrophus* associated to the hygromycine B resistance gene from *Escherichia coli* was amplified by PCR with the oligonucleotides

P1 5' atgaagcttggggtttgagggccaatggaacgaaactagtgtaccacttgacc 3' and

P2 5'gacagatctggcgccattcgccattcag 3'

using pGUS5 as template (Mönke, E. and Schäfer, W.; 1993, Mol. Gen. Genet. 241: 73-80). The PCR is done using standard protocols; e.g. as described in Maniatis et al., Mol. Cloning.

The resulting DNA fragment was inserted in the plasmid pFDX3809 (WO 01/38504) by the restriction site Hind III and Bgl II introduced by the oligonucleotides P1 and P2. The resulting plasmid pHygB serves as template for a further PCR, wherein the Oligonucleotides

ANK 518 5' ggaatcggtcaatacactac 3'

ANK 519 5' tgtagatctctattcctttgccctcggacgagt 3'

are used to shorten the hygromycin B resistance gene specifically. The resulting PCR fragment comprising 575 bp of the 3' end of the hygromycine gene was inserted in the plasmid pHygB via the restriction sites Nde I/ Bgl II generating the plasmid pHygB-NOS.

A Hind III / Ssp I DNA fragment of 2019 bp containing the expression cassette GPD1 promoter, the hygromycine B resistance gene and the nopaline synthase terminator was isolated from pHygB-NOS and inserted in the pUCmini plasmid previously treated with EcoRI and HindIII restriction enzymes to give the plasmid pUCmini-Hyg; to do so, the EcoRI ends were was made compatible with Ssp I by a fill-in treatment using the Klenow fragment of DNA polymerase I. A second version of pUCmini-Hyg, called pUCmini-Hyg-TA, was obtained by the insertion of the following adaptor in the NotI/ AscI restriction sites of pUCmini-Hyg:

5' GGCCGCCACGGATATCTTGGCCAAAGAATTCCTGG 3'

3' CGGTGCCTATAGAACCGGTTTCTTAAGGACCGCGC 5'

The adaptor contains 2 XcmI restriction sites so that XcmI digest of pUCmini-Hyg-TA creates T-overhangs that permits direct cloning of PCR products made with the classical Taq-polymerases.

Example 2 - Construction of a vector comprising PKS

SEQ ID NO: PKS was amplified by PCR with degenerated primers

LC1 5'-GA($^{T}/_{C}$) CCI ($^{A}/_{C}$)GI TT($^{T}/_{C}$) TT($^{T}/_{C}$) AA($^{T}/_{C}$) ATG-3'

LC2c 5'-GTI CCI GTI CC($^{A}/_{G}$) TGC AT($^{T}/_{C}$) TC-3'

based on the conserved amino acid sequence of the PKS gene sequences from Aspergillus nidulans, Colletotrichum lagenarium, Penicillium patulum, and Aspergillus parasiticus (Bingle et al., 1999). Thermal cycling parameters consisted of an initial denaturation at 94°C for 3 min followed by 34 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (annealing), 72°C for 3 min (extension) and a final extension at 72°C for 10 min according to standard procedures. The resulting PCR product was cloned into the pGEM-T vector (Promega, Mannheim, Germany) to give the plasmid pGEM-T/PKS833 and sequenced. A 633 bp DNA fragment (2236bp to 2870bp of SEQ ID NO:1) was amplified by PCR using the oligonucleotides

ANK593 5' ATAAGAATGCGGCCGCAATGGCCCTCGAAACAGC 3'

ANK594 5' AAATGGCGCGCCGCGCCCAGAATGACACC 3'

and cloned into the plasmid pUCmini-Hyg using the restriction site NotI and AscI present in the oligonucleotide sequences. The resulting plasmid pUCmini-Hyg-PKS is used for homologous recombination.

The flanking regions of the putative PKS DNA fragment were obtained by inverse PCR (Triglia T, Peterson MG, Kemp DJ, Nucleic Acids Res 1988 Aug 25;16(16):8186). Genomic DNA was treated with the restriction enzymes PstI, NcoI, or XhoI respectively. DNA was then self-ligated to get circular DNA molecule. The latter was used as template for the inverse PCR reaction using the primers P1A: 5' TGCCACCTGTAGTCTGCAATCAG 3'and P2A:5' TGACTAACCCTGACAACTTCGCTG 3' deduced from the putative PKS DNA fragment of the plasmid pGEM-T/PKS833 described above.

In a second step, the PCR product was reamplified with the nested primers P1B:5' CCAGGATCCGACTGCTCAG 3' and P2A:5'CTACATCGAGATGCACGGCAC 3' (deduced from the PKS DNA fragment of the plasmid pGEM-T/PKS833) , cloned into the pCR-XL-TOPO vector (Invitrogen) and sequenced. As result we got the two flanking regions of the

known PKS fragment linked on a PstI, NcoI or XhoI restriction site, respectively set forth in SEQ ID NO:1.

Example 3 Transformation of F. graminearum

50 ml of CM-medium (Leach et al., 1982, J. Gen. Microbiol. 128: 1719-1729) were inoculated with approximately 10^5 conidia, and incubated for 2 days at 28°C, 140 rpm. Resulting hyphae were homogenized in a Warring-Blender; 200 ml CM were inoculated with 10 ml hyphal suspension, and incubated overnight at 24°C. Mycel were trapped on a sterile filter, and washed two times with sterile water. 2 g of the hyphae were resuspended in 20 ml Driselase/Glucanase (Interspex Products, San Maneo, USA; 5% / 3% in 700 mM NaCl, pH 5.6), and digested 2½ to 3 h at 28°C, 75 rpm. Undigested hyphal were removed from the protoplast suspension by filtration through gauze and Nybold membrane (50 µm pore size). The protoplast suspension were combined with 700 mM NaCl and again passed through the gauze and the Nybold membrane. The protoplasts were pelleted by centrifugation (1300 x g) in a swing-out Rotor and washed two times with ice-cold NaCl 700 mM and centrifuge (830 x g). Then the protoplasts were resuspended in STC (0.8 M sorbitol, 50 mM Tris-HCl pH 8.0, 50 mM $CaCl_2$) and store on ice until transformation (maximal 1 week).

For transformation, protoplasts were resuspended in 4 parts STC and 1 part SPTC (0.8 M sorbitol, 40% polyethylene glycol 4000, 50 mM Tris-HCl pH 8.0, 50 mM $CaCl_2$) at a concentration of 0.5-2 x 10^8/ml; 30 µg of the pUCmini-Hyg-PKS plasmid DNA linearized with the *Eco*47III restriction site inside the PKS fragment and 5 µl heparin (5 mg/ml in STC) were added to 100 µl of the protoplast suspension in 10 ml tubes. After mixing, samples were incubated on ice for 30 min. 1 ml SPTC was mixed to the suspension and incubated at room temperature for 20 min. Protoplasts were mixed gently into 200 ml regeneration medium (0.1% (w/v) yeast extract, 0.1% (w/v) caseinhydrolysate, 34.2% (w/v) sucrose, 1.6% (w/v) granulated Agar) at 43°C and spread on a 94 mm plates (20 ml per plate). The plates were incubated at 28°C. After 12-24 h, the plates were overlaid with 10 ml per plate water based selective medium (16g/l granulated agar, 100mg/l Hygromycin and further incubated at 28°C until transformants were obtained, which were transferred to fresh CM-Hyg-plates (consisting of CM-media, 100 µg/ml hygromycin and 2% (w/v) Agar. The transformants were isolated by single spore isolation. For generation of conidia, the transformants were cultivated on SNA plates (Nirenberg, 1981, Canadian J. Botany 59: 1599-1609) under UV-light 7-14 days at 18°C. Dilutions of conidia were plated on CM-Hyg plates, and single

colonies were transferred from these plates to fresh CM-Hyg plates.

Example 4 Southernblot analysis

Genomic DNA was isolated from frozen hyphal material using the Puregene Genomic DNA Isolation Kit (Gentra Systems, Minneapolis USA) and digested for 6 h with *Nru*I restriction enzyme. The genomic DNA was separated by electrophoresis on a 1% (w/v) agarose gel and blotted onto a nylon membrane (Hybond NX; Amersham Pharmacia Biotech, Buckinghamshire England). A digoxigenin labeled probe was generated by PCR based on specific primers PKS forward 5'-GCG CTT GAG ATG GCT AGT ATC G-3' and and PKS reverse 5'-GTG CCG TGC ATC TCG ATG TAG-3' using pGEM-T/PKS833 as template and digoxigenin labeled dUTPs by PCR reaction according to the recommendation of the manufacturer(Roche Diagnostics GmbH, Mannheim). PCR conditions were 94°C for 3 min (initial denaturation) followed by 30 cycles of 94°C for 30 sec (denaturation), 55°C for 45 sec (annealing), 72°C for 1 min (extension) and a final extension at 72°C for 10 min. The non-radioactive hybridization and the detection were done under highly stringent conditions as described in Roche Molecular Biochemicals DIG Application Manual for Filter Hybridization (Roche Diagnostics GmbH, Mannheim).

To confirm the insertion of the vector construct into the PKS locus in comparison with the wild type gene, primers

EF-PKS 5' atgtctccaaaggaagctgagc 3'

ER-PKS 5'tcgagtgatggatactgcttcg 3'

are constructed based on the PKS DNA sequence from the plasmid pGEM-T/PKS833; four universal primers are constructed, wherein

Lac 92 5' cggctacactagaaggacagtatttggta 3'

Lac 93 5' gtcaggcaactatggatgaacgaaatagac 3'

Lac 94 5' acccatctcataaataacgtcatgc 3'; and

Lac 95 5' caactctatcagagcttggttga 3'

permit amplification of a 412 bp DNA fragment of the hygromycin cassette.

PCR reactions were conducted in classical conditions: 94°C for 3 min (initial denaturation) followed by 30 cycles of 94°C 60 sec (denaturation), 55°C for 90 sec (annealing), 72°C for 90 sec (extension) and a final extension at 72°C for 10 min.

6 recombinant clones resistant to Hygromycine were analyzed by PCR using the primer set Lac 94 / Lac 95 specific for the hygromycin resistance gene. All the mutants were found to present the expected DNA fragment of 412 bp indicating the integration of the plasmid pUCmini-Hyg-PKS in the genome.

A 712 bp corresponding to the PKS gene could be amplified with the primer set EF-PKS/ER-PKS mentioned above using genomic DNA from a wild type strain; on the contrary no PCR fragment were amplified with genomic DNA from the recombinant clones indicating that the PKS gene is disrupted by the insertion of pUCmini-Hyg-PKS. This was confirmed by PCR amplification EF-PKS combined with Lac 93 (hybridizing to the plasmid backbone near Not I restriction site) and ER-PKS combined with Lac 92 (hybridizing to the plasmid backbone near Asc I restriction site). In both cases, DNA fragments of about 600 bp were amplified for the recombinant clones but not for the wild type strain (WT). All together the PCR analysis using the different primer sets proves that the plasmid pUCmini-Hyg-PKS was targeted specifically in the PKS locus by homologous recombination. This process permits to disrupt the PKS gene since the recombinant mutants were found to lack the typical pigmentation (purple) of the wildtype strain.

Brief description of the figures

Figure 1: Map of pUCmini-Hyg

Figure 1: Map of PUCmini-Hyg TA

Claims

1. A plasmid vector for targeted transformation of filamentous fungi comprising

   a) an origin of replication for a host organism which is not originating from the filamentous fungi to be transformed;

   b) a selection marker for a host organism not originating from the filamentous fungi;

   c) a promotor facilitating recombinant expression in fungi that is functionally linked to the coding region of the hygromycin resistance gene which is functionally linked to a terminator which facilitates transcription termination in filamentous fungi;

   wherein the overall size of the elements a), b) and c) does not exceed 4500 bp; and

   d) a nucleic acid sequence, which is homologous to nucleic acid sequences of the target organism and makes homologous recombination in the filamentous fungi possible.

2. A plasmid vector as claimed in claim 1, wherein the an origin of replication a) originates from bacteria.

3. A plasmid vector as claimed in claim 1 to 2, wherein the selection marker b) imparts a resistance to antibiotics.

4. A plasmid vector according to claim 1 to 3, wherein the promotor of element c) is selected from the group consisting of the GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5-, AOX1, GAL10/CYC-1, CYC1, OliC-, ADH-, TDH-, Kex2-, MFa- and the NMT-promotor.

5. A plasmid vector according to claim 1 to 4, wherein the terminator of element c) is selected from the group consisting of the AOX1-, nos-, PGK-, TrpC- and the CYC1-terminator.

6. A plasmid vector according to claim 1 to 2, wherein the promotor of element c) is the GPD-1-promotor and the terminator of element c) is the nos-terminator.

7. A plasmid vector according to claims 1 to 6, wherein the nucleic acid sequence d) is functionally linked to a promotor facilitating recombinant expression in filamentous fungi.

8. A plasmid vector according to claims 1 to 7, wherein the nucleic acid sequence d) is functionally linked to a transcription terminator facilitating recombinant expression in filamentous fungi.

9. A selection marker comprising a nucleic acid sequence encoding a polyketide snythetase fragment, wherein said nucleic acid sequence comprises

i. a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2; or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14 by back translation; or

iii. functional equivalents of the nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by back translation from a functional equivalent of an amino acid sequence shown in SEQ ID NO:4 that has at least an identity of 39% with the SEQ ID NO:4 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 41% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 49% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of 58% with the SEQ ID NO:10 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:12 that has at least an identity of 39% with the SEQ ID NO:12 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:14 that has at least an identity of 46% with the SEQ ID NO:14; or

iv. functional equivalents of the nucleic acid sequence shown in SEQ ID NO:1 having at least an identity of 80% with the SEQ ID NO:1 or functional equivalents of SEQ ID NO:2 having at least an identity of 80% with the SEQ ID NO:2; or

v. parts of the nucleic acid sequence as defined in i), ii), iii) or iv) consisting of at least 300bp.

10. Use of a nucleic acid sequence as marker for targeted transformation in filamentous fungi said nucleic acid sequence comprising

i. a nucleic acid sequence according to claim 9;

ii. functional equivalents of the nucleic acid sequence according to claim 9 which, owing to the degeneracy of the genetic code, can be deduced by back translation from a functional equivalent of an amino acid sequence shown in SEQ ID NO:4 that has at least an identity of 37% with the SEQ ID NO:4 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 38% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 39% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of 48% with the SEQ ID NO:10 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:12 that has at least an identity of 36% with the SEQ ID NO:12 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:14 that has at least an identity of 32% with the SEQ ID NO:14; or

iii. parts of the nucleic acid sequence as defined in ii) consisting of at least 300bp.

11. A plasmid vector for targeted transformation of filamentous fungi additionally comprising a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment said nucleic acid sequence comprising

i. a nucleic acid sequence according to claim 9;

ii. functional equivalents of the nucleic acid sequence according to claim 9 which, owing to the degeneracy of the genetic code, can be deduced by back translation from a functional equivalent of an amino acid sequence shown in SEQ ID NO:4 that has at least an identity of 37% with the SEQ ID NO:4 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 38% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8

that has at least an identity of 39% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of 48% with the SEQ ID NO:10 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:12 that has at least an identity of 36% with the SEQ ID NO:12 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:14 that has at least an identity of 32% with the SEQ ID NO:14; or

iii. parts of the nucleic acid sequence as defined in ii) consisting of at least 300bp.

12. A plasmid vector for targeted transformation of filamentous fungi as claimed in claims 1 to 8, additionally comprising a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment said nucleic acid sequence comprising

i. nucleic acid sequence according to claim 9;

ii. functional equivalents of the nucleic acid sequence according to claim 9 which, owing to the degeneracy of the genetic code, can be deduced by back translation from a functional equivalent of an amino acid sequence shown in SEQ ID NO:4 that has at least an identity of 37% with the SEQ ID NO:4 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 38% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 39% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of 48% with the SEQ ID NO:10 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:12 that has at least an identity of 36% with the SEQ ID NO:12 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:14 that has at least an identity of 32% with the SEQ ID NO:14; or

iii. parts of the nucleic acid sequence as defined in ii) consisting of at least 300bp.

13. A method for preparing mutated filamentous fungi, comprising the following steps

a) transferring a plasmid vector according to claim 11 or 12 into a filamentous fungi;

b) selecting successfully transformed filamentous fungi by the absence of colour.

14. A method as claimed in claim 13, wherein the plasmid vector comprises at least an additional selection marker.

15. A method as claimed in claims 12 to 14, wherein the selection is confirmed by PCR.

16. A method as claimed in claims 12 to 15, wherein the filamentous fungi are succesuflly transformed and identified in a high-throughput screening.

Plasmid vectors for transformation of filamentous fungi

Abstract

The invention relates to novel plasmid vectors for transformation of filamentous fungi and to a method of modifiying the genome of filamentous fungi based on these vectors. The invention furthermore relates to the modification of a specific gene via the process of homologous recombination, to recombinant expression of foreign genes in filamentous fungi and to new selection markers for detecting succesfull transfer of the target gene.

SEQUENCE LISTING

<110> BASF Aktiengesellschaft

<120> Plasmid vectors for transformation of filamentous fungi

<130> 20020174

<140>
<141>

<160> 14

<170> PatentIn Ver. 2.1

<210> 1
<211> 5888
<212> DNA
<213> Fusarium graminearum

<400> 1
atcgagtttc gtgttgcgtg tcatggtgta gcgaacaata ccatactcct ccatcaaagg 60
gctgacgagc ttggcatgga cctttgtcat atggtgcctg tactcttttct cgctaagaga 120
aggtttttg taggccgtga tggttaggca aacaagggta tcaggttgac cggtgacagt 180
tgcctcatga gaggtttctt gggtatcact atgaggcgac atgatgcagg ttggagtttg 240
ttatcgaagt gctgggtagg atttggttga cagagttgca taaactggag ctctggatac 300
gcaaggggtc ataagagtat ggatacgccg tcgattagaa gctgtcagat ccagtgcatc 360
aatggcccga atgacgagag attgctgctc ggcactgaaa gtctgcagcg tgcttgtttc 420
ggagttatct ctacagctgc accgaatgac gaacaagccc gactatccat gtccgacgag 480
cccggaccga cgtgatccga atgctgtcta acacttacca agacaatcat ggggtatctc 540
tgaattatga ttgtgttgct caagcagcta atcccaactc ttaatcacgt caagtgccat 600
gcttacctgt attatcatct gtcatcaaca taccaactcc tatatatagg ggattgtgct 660
tcattacctc tccagtaaca actacttgga gttcaaacca catttcaact tactctttta 720
gttcattcaa catgacccca tcaatgatgg aggtattcgt ttttggggac caaagcacac 780
gctttgcccc tccactgaaa gacctactcc tcaaaggcaa cagtccttac ttgacacatt 840
ttgttaaaca agttcacgca cttcttagaa gggagatatc atccttgccg gcagttcaac 900
agaagctttt cccaaacttt gccgacattc aggaactcgt ctccaagtca gattggggca 960
gtggtaaccc tgctttgaca agcgctttag catgctttta ccatctttgc agtttcattc 1020
agtgagtaac ataaccaagt acccatagat ctctcctgac catccagcct tttacgatgg 1080
acaaggtcgt accttcctt cggagaacag tcgcattatt ggactttgcg ttggttcact 1140
cgctgctact gctgtcagtt gctccacatc actgagtgaa ttggtatcag ctggtgtaga 1200
tgctgttcgt gtggcattgc acgtcggact acgggtatgg cgaactacct ccctttcga 1260
tgtaccagac aggccctccg ccacttggtt cataattgtg cccgaggcag tactaccaag 1320
agaatctgcg caagaccgac ttgactcatt catcattgaa atggtaagca attctttgtc 1380
cttggagctc tcataaaaca tattcactct tctttaggga cttgctcgat catcagttcc 1440
ttacatcagc tcggtcgcac atcacaacat gaccatcagt ggtccaccat ccgtcctcga 1500
aaagttcatt cacagtatat caacatcacc gaaagattct cttccagtgc cgatctatgc 1560
tccgtaccac gccagccatc tttacagcat ggatgatgta gacgaggtcc ttagcctgtc 1620
tgcaccttct tttgcatcag agtccatcat tccactcatt tcaagctcct cgggtgacga 1680
gttacagcca ctcaagtatg cagatctact ccgctgctgt gttagtgata tgctcataca 1740
gccactggat cttaccaagg tctcacaagc agtggcccag cttctcgagg ttagctcatc 1800
tacacgtgcc ataataaagc ctatagcaac cagcgtctcc aacagtctag tgtctgtttt 1860
ggagccgacg ctagcagaac gatgcgccgt ggacaacagc atggggccca aagcctcgac 1920

cagccactca tcagcagaga cacaaaccga gtcatcaagc aagaactcca aaattgcgat 1980
tgttgctatg tctggtcgct ttccagacgc agctgacttg agtgaattct gggatcttct 2040
ctacgaaggt cgcgatgttc atcgacaaat tcccgaggac cgattcaacg cagagctcca 2100
ttacgacgct actgggcgac gtaagaacac cagcaaggtc atgaatggct gcttcatcaa 2160
ggaaccagga ctgttcgacg ctaggttctt caacatgtct ccaaaggaag ctgagcagtc 2220
ggatcctggc cagcgaatgg ccctcgaaac agcttacgag gcgcttgaga tggctagtat 2280
cgtaccagac agaacacctt cgacacagag agatcgtgtt ggtgtgttct acggcatgac 2340
tagcgatgat tggagagagg tcaacagtgg gcagaatgtc gacacttatt ttattcctgg 2400
taagttgttt gttcgcgttt cacgacaata ctgattgcag actacaggtg gcaacagagc 2460
gttcactcct ggtcgactca actacttctt caagttcagt gggcctagcg ctagtgttga 2520
tacggcttgc tcctccagtc tcgttggctt gcacttggct tgtaattccc tctggagaaa 2580
tgattgcgat acagctattg cgggcggaac caatgtcatg actaaccctg acaacttcgc 2640
tggtttggac cgaggccact tcctatctca agaaccggta agtctctcac agatcttgga 2700
agcttccttc tttttagtgt ttttactgac cttgttccag gcaactgcaa caccttgac 2760
gatggagcag acggatactg tcgagctgat ggcgtcggaa ccatcatcct caagcggctt 2820
gaggacgccg aagctgacaa tgaccctatt ctcggtgtca ttctgggcgc ttacacaaac 2880
cactcagccg aagcagtatc catcactcga ccacatgccg gagctcaaga gtacatcttc 2940
tccaaactcc tccgtgagtc gggcaccgat ccctacaacg ttagctacat cgagatgcac 3000
ggcacaggca ctcaagccgg cgacgcaacc gagatgacat ccgtcctcaa gacgtttgct 3060
cctaccagcg gcttcggcgg tcgattgcct caccaaaacc ttcacttggg ttcagtcaag 3120
gccaatgtcg ggcacggtga atccgcatct ggtatcattg ctctgatcaa gacgctgctt 3180
atgatggaga agaacatgat cccgccgcat tgtggtatca agacaaagat caatcaccat 3240
tttcctacgg atctcactca gcgcaatgtc catatcgcca aagttccgac atcttggaca 3300
agatcgggtc aagccaatcc acgcattgct ttcgtcaata acttctctgc cgctggtggt 3360
aactctgctg tcctactgca agatgctcct cagccatcgg tagtttcgga tgttacagac 3420
cctcgcacat cccatgttgt cactatgtcc gctcgatcag cagattccct caggaagaac 3480
ctcgccaatc tcaaggagct tgtagaaggc caaggtgact cggaggtcgg cttcctgagc 3540
aagctgtcct acacaaccac cgccaggcgc atgcatcatc aattccgagc ttcggtcaca 3600
gcacagactc gtgaacagct gctgaagggc cttgattccg ccattgaacg ccaggatgtg 3660
aagaggatcc ccgccgccgc gccctctgtc ggctttgtgt ttagcggcca aggcgcccaa 3720
taccgtggta tgggcaagga gtactttaca tctttcacag ccttccgctc tgagatcatg 3780
tcttacgaca gtatcgccca agcccaaggc ttcccgtcaa tcctcccact gatccgagga 3840
gaggtggaag ctgactcgtt gagtcctgtt gagatccagc tgggtctcac ttgcctgcag 3900
atggcactgg ccaagctatg gaagtcattc ggtgttgagc caggctttgt tctcggacac 3960
agcttaggcc actatgctgc tttacacgtc gctggtgttc tgtccgccaa tgataccatt 4020
tacctcactg gcatcagagc gcagctgctc gtggataagt gccaggcagg aacccactca 4080
atgctggcag tgagggcatc cttactacag atccaacagt tcctcgatgc caacattcac 4140
gaggttgcat gtgtcaatgg ttcacgagaa gtcgtcatca gtggacgcgt tgccgacatt 4200
gaccagctgg ttggcctttt gtcggctgac aacatcaagg cgacccgcgt caaggtgcca 4260
tttgccttcc actcagcgca ggttgacccc attctctccg acttggatac agcggcgtcg 4320
cgcgtcacct tccactcccc ccagattcct gttctttgtg ccccttgacag ctctgtcatc 4380
agccctggaa accatggtgt cattggtccc cttcatctac agcgacattg tcgtgagaca 4440
gtcaactttg agggtgctct acatgctgcg gagcacgaga agatcatcaa caagacatca 4500
actctatgga tcgagattgg tccccatgtt gtctgctcta ccttcctcaa gtccagcctt 4560
ggtccaagca cacctgctat cgcatcgctt cgccgaaatg acgattgctg gaaggtgttg 4620
gctgatggtt tgagcagtct ctacagcagc gggttgacaa ttgactagaa cgagtatcat 4680
cgcgacttca aggcctctca ccaggtactt cgtctgcctt gttacagctg ggagcacaag 4740
aattactgga tacagtacaa gtacgattgg tccttggcta aaggtgatcc tccaattgcc 4800
cctaacagct cggttgaagc agtctcagct ttatcaacac cctcggtcca gaagattcta 4860

caggagactt cccttgatca ggtattgact atcgttgctg agacagatct cgcgagccct 4920
ctattgtcag aggttgccca aggtcatcgg gtcaacggtg tcaaagtctg cacatcttcc 4980
gtgtacgctg atgttggctt gacgctgggt aagtacattt tggacaacta ccgcaccgac 5040
ttagagggtt atgcggtcga tgttcacggt attgaggtcc acaagccact tcttctcaaa 5100
gaagacatga acggaacgcc ccaggctaca ccgttccgca tcgaagtgcg atacccaatc 5160
cagagcacca cggcgctgat gagcatctcc accactggcc ccaacggtca gcacatcaag 5220
catgctaact gcgaacttcg actcgagcat ccgtcgcaat gggaagcgga gtgggatcgc 5280
caagcctacc tcatcaatcg cagcgtcaac tgccttctgc agcgatcagc acaaggtttg 5340
gacagcatgt tggcaaccgg aatggtctac aaggtcttct cctccctcgt cgactatgcc 5400
gatggctaca agggtctgca ggaggttgtc ttgcacagcc aagagctcga gggcacagca 5460
aaagtgcgct tccaaactcc ctcgggaggt ttcgtctgca atcccatgtg gattgacagc 5520
tgtggtcaga cgaccggctt catgatgaac tgtcatcaga ctacgcccaa tgactacgtc 5580
tacgtcaatc atggctggaa gtcgatgaga ttggccaagg cgttccgtga agatggtacc 5640
tatcggactt atatccggat gaggcccatt gatagcacca agttcgctgg tgacttgtac 5700
attcttgatg aggatgacac tgtggttggt gtttatggag acataacaat gagtaacacg 5760
aatctttgtc aacaacaatg ctaacgtaaa gaacagttcc aaggtttgcc gcgacgagtt 5820
ctcaacacag tcctgccatc tgccaacgcg gttccagttg atgctcccat ggttcaagac 5880
gacctcca 5888

<210> 2
<211> 5157
<212> DNA
<213> Fusarium graminearum

<400> 2
atgacccccat caatgatgga ggtattcgtt tttggggacc aaagcacacg ctttgccccc 60
ccactgaaag acctactcct caaaggcaac agtccttact tgacacattt tgttaaacaa 120
gttcacgcac ttcttagaag ggagatatca tccttgccgg cagttcaaca gaagctttcc 180
ccaaactttg ccgacattca ggaactcgtc tccaagtcag attggggcag tggtaaccct 240
gctttgacaa gcgctttagc atgcttttac catctttgca gtttcattca gtgagtaaca 300
taaccaagta cccatagatc tctcctgacc atccagcctt ttacgatgga caaggtcgta 360
cctttccttc ggagaacagt cgcattattg gactttgcgt tggttcactc gctgctactg 420
ctgtcagttg ctccacatca ctgagtgaat tggtatcagc tggtgtagat gctgttcgtg 480
tggcattgca cgtcggacta cgggtatggc gaactacctc ccttttcgat gtaccagaca 540
ggccctccgc cacttggttc ataattgtgc ccgaggcagt actaccaaga gaatctgcgc 600
aagaccgact tgactcattc atcattgaaa tggtaagcaa ttctttgtcc ttggagctct 660
cataaaacat attcactctt ctttagggac ttgctcgatc atcagttcct tacatcagct 720
cggtcgcaca tcacaacatg accatcagtg gtccaccatc cgtcctcgaa aagttcattc 780
acagtatatc aacatcaccg aaagattctc ttccagtgcc gatctatgct ccgtaccacg 840
ccagccatct ttacagcatg gatgatgtag acgaggtcct tagcctgtct gcaccttctt 900
ttgcatcaga gtccatcatt ccactcattt caagctcctc gggtgacgag ttacagccac 960
tcaagtatgc agatctactc cgctgctgtg ttagtgatat gctcatacag ccactggatc 1020
ttaccaaggt ctcacaagca gtggcccagc ttctcgaggt tagctcatct acacgtgcca 1080
taataaagcc tatagcaacc agcgtctcca acagtctagt gtctgttttg gagccgacgc 1140
tagcagaacg atgcgccgtg gacaacagca tggggcccaa agcctcgacc agccactcat 1200
cagcagagac acaaaccgag tcatcaagca agaactccaa aattgcgatt gttgctatgt 1260
ctggtcgctt tccagacgca gctgacttga gtgaattctg ggatcttctc tacgaaggtc 1320
gcgatgttca tcgacaaatt cccgaggacc gattcaacgc agagctccat tacgacgcta 1380
ctgggcgacg taagaacacc agcaaggtca tgaatggctg cttcatcaag gaaccaggac 1440
tgttcgacgc taggttcttc aacatgtctc caaaggaagc tgagcagtcg gatcctggcc 1500
agcgaatggc cctcgaaaca gcttacgagg cgcttgagat ggctagtatc gtaccagaca 1560

gaacaccttc gacacagaga gatcgtgttg gtgtgttcta cggcatgact agcgatgatt 1620
ggagagaggt caacagtggg cagaatgtcg acacttattt tattcctggt aagttgtttg 1680
ttcgcgtttc acgacaatac tgattgcaga ctacaggtgg caacagagcg ttcactcctg 1740
gtcgactcaa ctacttcttc aagttcagtg ggcctagcgc tagtgttgat acggcttgct 1800
cctccagtct cgttggcttg cacttggctt gtaattccct ctggagaaat gattgcgata 1860
cagctattgc gggcggaacc aatgtcatga ctaaccctga caacttcgct ggtttggacc 1920
gaggccactt cctatctcaa gaaccggtaa gtctctcaca gatcttggaa gcttccttct 1980
ttttagtgtt tttactgacc ttgttccagg caactgcaac acctttgacg atggagcaga 2040
cggatactgt cgagctgatg gcgtcggaac catcatcctc aagcggcttg aggacgccga 2100
agctgacaat gaccctattc tcggtgtcat tctgggcgct tacacaaacc actcagccga 2160
agcagtatcc atcactcgac cacatgccgg agctcaagag tacatcttct ccaaactcct 2220
ccgtgagtcg ggcaccgatc cctacaacgt tagctacatc gagatgcacg gcacaggcac 2280
tcaagccggc gacgcaaccg agatgacatc cgtcctcaag acgtttgctc ctaccagcgg 2340
cttcggcggt cgattgcctc accaaaacct tcacttgggt tcagtcaagg ccaatgtcgg 2400
gcacggtgaa tccgcatctg gtatcattgc tctgatcaag acgctgctta tgatggagaa 2460
gaacatgatc ccgccgcatt gtggtatcaa gacaaagatc aatcaccatt ttcctacgga 2520
tctcactcag cgcaatgtcc atatcgccaa agttccgaca tcttggacaa gatcgggtca 2580
agccaatcca cgcattgctt tcgtcaataa cttctctgcc gctggtggta actctgctgt 2640
cctactgcaa gatgctcctc agccatcggt agtttcggat gttacagacc ctcgcacatc 2700
ccatgttgtc actatgtccg ctcgatcagc agattccctc aggaagaacc tcgccaatct 2760
caaggagctt gtagaaggcc aaggtgactc ggaggtcggc ttcctgagca agctgtccta 2820
cacaaccacc gccaggcgca tgcatcatca attccgagct tcggtcacag cacagactcg 2880
tgaacagctg ctgaagggcc ttgattccgc cattgaacgc caggatgtga agaggatccc 2940
cgccgccgcg cccctctgtcg gctttgtgtt tagcggccaa ggcgcccaat accgtggtat 3000
gggcaaggag tactttacat ctttcacagc cttccgctct gagatcatgt cttacgacag 3060
tatcgcccaa gcccaaggct tcccgtcaat cctcccactg atccgaggag aggtggaagc 3120
tgactcgttg agtcctgttg agatccagct gggtctcact tgcctgcaga tggcactggc 3180
caagctatgg aagtcattcg gtgttgagcc aggctttgtt ctcggacaca gcttaggcca 3240
ctatgctgct ttacacgtcg ctggtgttct gtccgccaat gataccattt acctcactgg 3300
catcagagcg cagctgctcg tggataagtg ccaggcagga acccactcaa tgctggcagt 3360
gagggcatcc ttactacaga tccaacagtt cctcgatgcc aacattcacg aggttgcatg 3420
tgtcaatggt tcacgagaag tcgtcatcag tggacgcgtt gccgacattg accagctggt 3480
tggccttttg tcggctgaca acatcaaggc gacccgcgtc aaggtgccat ttgccttcca 3540
ctcagcgcag gttgacccca ttctctccga cttggataca gcggcgtcgc gcgtcacctt 3600
ccactccctc cagattcctg ttctttgtgc ccttgacagc tctgtcatca gccctggaaa 3660
ccatggtgtc attggtcccc ttcatctaca gcgacattgt cgtgagacag tcaactttga 3720
gggtgctcta catgctgcgg agcacgagaa gatcatcaac aagacatcaa ctctatggat 3780
cgagattggt ccccatgttg tctgctctac cttcctcaag tccagccttg gtccaagcac 3840
acctgctatc gcatcgcttc gccgaaatga cgattgctgg aaggtgttgg ctgatggttt 3900
gagcagtctc tacagcagcg ggttgacaat tgactagaac gagtatcatc gcgacttcaa 3960
ggcctctcac caggtacttc gtctgccttg ttacagctgg gagcacaaga attactggat 4020
acagtacaag tacgattggt ccttggctaa aggtgatcct ccaattgccc ctaacagctc 4080
ggttgaagca gtctcagctt tatcaacacc ctcggtccag aagattctac aggagacttc 4140
ccttgatcag gtattgacta tcgttgctga gacagatctc gcgagccctc tattgtcaga 4200
ggttgcccaa ggtcatcggg tcaacggtgt caaagtctgc acatcttccg tgtacgctga 4260
tgttggcttg acgctgggta agtacatttt ggacaactac cgcaccgact tagagggtta 4320
tgcggtcgat gttcacggta ttgaggtcca caagccactt cttctcaaag aagacatgaa 4380
cggaacgccc caggctacac cgttccgcat cgaagtgcga tacccaatcc agagcaccac 4440
ggcgctgatg agcatctcca ccactggccc caacggtcag cacatcaagc atgctaactg 4500

```
cgaacttcga ctcgagcatc cgtcgcaatg ggaagcggag tgggatcgcc aagcctacct 4560
catcaatcgc agcgtcaact gccttctgca gcgatcagca caaggtttgg acagcatgtt 4620
ggcaaccgga atggtctaca aggtccttct ctccctcgtc gactatgccg atggctacaa 4680
gggtctgcag gaggttgtct tgcacagcca agagctcgag ggcacagcaa aagtgcgctt 4740
ccaaactccc tcgggaggtt tcgtctgcaa tcccatgtgg attgacagct gtggtcagac 4800
gaccggcttc atgatgaact gtcatcagac tacgcccaat gactacgtct acgtcaatca 4860
tggctggaag tcgatgagat tggccaaggc gttccgtgaa gatggtacct atcggactta 4920
tatccggatg aggcccattg atagcaccaa gttcgctggt gacttgtaca ttcttgatga 4980
ggatgacact gtggttggtg ttatggaga cataacaatg agtaacacga atctttgtca 5040
acaacaatgc taacgtaaag aacagttcca aggtttgccg cgacgagttc tcaacacagt 5100
cctgccatct gccaacgcgg ttccagttga tgctcccatg gttcaagacg acctcca    5157
```

<210> 3
<211> 291
<212> DNA
<213> Fusarium graminearum

<220>
<221> CDS
<222> (1)..(291)

<400> 3

```
atg acc cca tca atg atg gag gta ttc gtt ttt ggg gac caa agc aca   48
Met Thr Pro Ser Met Met Glu Val Phe Val Phe Gly Asp Gln Ser Thr
 1               5                  10                  15

cgc ttt gcc cct cca ctg aaa gac cta ctc ctc aaa ggc aac agt cct   96
Arg Phe Ala Pro Pro Leu Lys Asp Leu Leu Leu Lys Gly Asn Ser Pro
            20                  25                  30

tac ttg aca cat ttt gtt aaa caa gtt cac gca ctt ctt aga agg gag   144
Tyr Leu Thr His Phe Val Lys Gln Val His Ala Leu Leu Arg Arg Glu
        35                  40                  45

ata tca tcc ttg ccg gca gtt caa cag aag ctt ttc cca aac ttt gcc   192
Ile Ser Ser Leu Pro Ala Val Gln Gln Lys Leu Phe Pro Asn Phe Ala
    50                  55                  60

gac att cag gaa ctc gtc tcc aag tca gat tgg ggc agt ggt aac cct   240
Asp Ile Gln Glu Leu Val Ser Lys Ser Asp Trp Gly Ser Gly Asn Pro
65                  70                  75                  80

gct ttg aca agc gct tta gca tgc ttt tac cat ctt tgc agt ttc att   288
Ala Leu Thr Ser Ala Leu Ala Cys Phe Tyr His Leu Cys Ser Phe Ile
                85                  90                  95

cag                                                               291
Gln
```

<210> 4
<211> 97
<212> PRT
<213> Fusarium graminearum

<400> 4

Met Thr Pro Ser Met Met Glu Val Phe Val Phe Gly Asp Gln Ser Thr
1 5 10 15

Arg Phe Ala Pro Pro Leu Lys Asp Leu Leu Leu Lys Gly Asn Ser Pro
20 25 30

Tyr Leu Thr His Phe Val Lys Gln Val His Ala Leu Leu Arg Arg Glu
35 40 45

Ile Ser Ser Leu Pro Ala Val Gln Gln Lys Leu Phe Pro Asn Phe Ala
50 55 60

Asp Ile Gln Glu Leu Val Ser Lys Ser Asp Trp Gly Ser Gly Asn Pro
65 70 75 80

Ala Leu Thr Ser Ala Leu Ala Cys Phe Tyr His Leu Cys Ser Phe Ile
85 90 95

Gln

<210> 5
<211> 300
<212> DNA
<213> Fusarium graminearum

<220>
<221> CDS
<222> (1)..(300)

<400> 5

```
gcc ttt tac gat gga caa ggt cgt acc ttt cct tcg gag aac agt cgc   48
Ala Phe Tyr Asp Gly Gln Gly Arg Thr Phe Pro Ser Glu Asn Ser Arg
 1               5                  10                  15

att att gga ctt tgc gtt ggt tca ctc gct gct act gct gtc agt tgc   96
Ile Ile Gly Leu Cys Val Gly Ser Leu Ala Ala Thr Ala Val Ser Cys
            20                  25                  30

tcc aca tca ctg agt gaa ttg gta tca gct ggt gta gat gct gtt cgt   144
Ser Thr Ser Leu Ser Glu Leu Val Ser Ala Gly Val Asp Ala Val Arg
        35                  40                  45

gtg gca ttg cac gtc gga cta cgg gta tgg cga act acc tcc ctt ttc   192
Val Ala Leu His Val Gly Leu Arg Val Trp Arg Thr Thr Ser Leu Phe
    50                  55                  60

gat gta cca gac agg ccc tcc gcc act tgg ttc ata att gtg ccc gag   240
Asp Val Pro Asp Arg Pro Ser Ala Thr Trp Phe Ile Ile Val Pro Glu
65                  70                  75                  80

gca gta cta cca aga gaa tct gcg caa gac cga ctt gac tca ttc atc   288
Ala Val Leu Pro Arg Glu Ser Ala Gln Asp Arg Leu Asp Ser Phe Ile
                85                  90                  95
```

att gaa atg gta 300
Ile Glu Met Val
100

<210> 6
<211> 100
<212> PRT
<213> Fusarium graminearum

<400> 6
Ala Phe Tyr Asp Gly Gln Gly Arg Thr Phe Pro Ser Glu Asn Ser Arg
1 5 10 15

Ile Ile Gly Leu Cys Val Gly Ser Leu Ala Ala Thr Ala Val Ser Cys
20 25 30

Ser Thr Ser Leu Ser Glu Leu Val Ser Ala Gly Val Asp Ala Val Arg
35 40 45

Val Ala Leu His Val Gly Leu Arg Val Trp Arg Thr Thr Ser Leu Phe
50 55 60

Asp Val Pro Asp Arg Pro Ser Ala Thr Trp Phe Ile Ile Val Pro Glu
65 70 75 80

Ala Val Leu Pro Arg Glu Ser Ala Gln Asp Arg Leu Asp Ser Phe Ile
85 90 95

Ile Glu Met Val
100

<210> 7
<211> 984
<212> DNA
<213> Fusarium graminearum

<220>
<221> CDS
<222> (1)..(984)

<400> 7
gga ctt gct cga tca tca gtt cct tac atc agc tcg gtc gca cat cac 48
Gly Leu Ala Arg Ser Ser Val Pro Tyr Ile Ser Ser Val Ala His His
1 5 10 15

aac atg acc atc agt ggt cca cca tcc gtc ctc gaa aag ttc att cac 96
Asn Met Thr Ile Ser Gly Pro Pro Ser Val Leu Glu Lys Phe Ile His
20 25 30

agt ata tca aca tca ccg aaa gat tct ctt cca gtg ccg atc tat gct 144
Ser Ile Ser Thr Ser Pro Lys Asp Ser Leu Pro Val Pro Ile Tyr Ala
35 40 45

ccg tac cac gcc agc cat ctt tac agc atg gat gat gta gac gag gtc 192
Pro Tyr His Ala Ser His Leu Tyr Ser Met Asp Asp Val Asp Glu Val
50 55 60

```
ctt agc ctg tct gca cct tct ttt gca tca gag tcc atc att cca ctc   240
Leu Ser Leu Ser Ala Pro Ser Phe Ala Ser Glu Ser Ile Ile Pro Leu
 65                  70                  75                  80

att tca agc tcc tcg ggt gac gag tta cag cca ctc aag tat gca gat   288
Ile Ser Ser Ser Ser Gly Asp Glu Leu Gln Pro Leu Lys Tyr Ala Asp
                 85                  90                  95

cta ctc cgc tgc tgt gtt agt gat atg ctc ata cag cca ctg gat ctt   336
Leu Leu Arg Cys Cys Val Ser Asp Met Leu Ile Gln Pro Leu Asp Leu
            100                 105                 110

acc aag gtc tca caa gca gtg gcc cag ctt ctc gag gtt agc tca tct   384
Thr Lys Val Ser Gln Ala Val Ala Gln Leu Leu Glu Val Ser Ser Ser,
        115                 120                 125

aca cgt gcc ata ata aag cct ata gca acc agc gtc tcc aac agt cta   432
Thr Arg Ala Ile Ile Lys Pro Ile Ala Thr Ser Val Ser Asn Ser Leu
    130                 135                 140

gtg tct gtt ttg gag ccg acg cta gca gaa cga tgc gcc gtg gac aac   480
Val Ser Val Leu Glu Pro Thr Leu Ala Glu Arg Cys Ala Val Asp Asn
145                 150                 155                 160

agc atg ggg ccc aaa gcc tcg acc agc cac tca tca gca gag aca caa   528
Ser Met Gly Pro Lys Ala Ser Thr Ser His Ser Ser Ala Glu Thr Gln
                165                 170                 175

acc gag tca tca agc aag aac tcc aaa att gcg att gtt gct atg tct   576
Thr Glu Ser Ser Ser Lys Asn Ser Lys Ile Ala Ile Val Ala Met Ser
            180                 185                 190

ggt cgc ttt cca gac gca gct gac ttg agt gaa ttc tgg gat ctt ctc   624
Gly Arg Phe Pro Asp Ala Ala Asp Leu Ser Glu Phe Trp Asp Leu Leu
        195                 200                 205

tac gaa ggt cgc gat gtt cat cga caa att ccc gag gac cga ttc aac   672
Tyr Glu Gly Arg Asp Val His Arg Gln Ile Pro Glu Asp Arg Phe Asn
    210                 215                 220

gca gag ctc cat tac gac gct act ggg cga cgt aag aac acc agc aag   720
Ala Glu Leu His Tyr Asp Ala Thr Gly Arg Arg Lys Asn Thr Ser Lys
225                 230                 235                 240

gtc atg aat ggc tgc ttc atc aag gaa cca gga ctg ttc gac gct agg   768
Val Met Asn Gly Cys Phe Ile Lys Glu Pro Gly Leu Phe Asp Ala Arg
                245                 250                 255

ttc ttc aac atg tct cca aag gaa gct gag cag tcg gat cct ggc cag   816
Phe Phe Asn Met Ser Pro Lys Glu Ala Glu Gln Ser Asp Pro Gly Gln
            260                 265                 270

cga atg gcc ctc gaa aca gct tac gag gcg ctt gag atg gct agt atc   864
Arg Met Ala Leu Glu Thr Ala Tyr Glu Ala Leu Glu Met Ala Ser Ile
        275                 280                 285
```

```
gta cca gac aga aca cct tcg aca cag aga gat cgt gtt ggt gtg ttc   912
Val Pro Asp Arg Thr Pro Ser Thr Gln Arg Asp Arg Val Gly Val Phe
    290                 295                 300

tac ggc atg act agc gat gat tgg aga gag gtc aac agt ggg cag aat   960
Tyr Gly Met Thr Ser Asp Asp Trp Arg Glu Val Asn Ser Gly Gln Asn
305                 310                 315                 320

gtc gac act tat ttt att cct ggt                                   984
Val Asp Thr Tyr Phe Ile Pro Gly
                325
```

<210> 8
<211> 328
<212> PRT
<213> Fusarium graminearum

<400> 8

```
Gly Leu Ala Arg Ser Ser Val Pro Tyr Ile Ser Ser Val Ala His His
 1               5                  10                  15

Asn Met Thr Ile Ser Gly Pro Pro Ser Val Leu Glu Lys Phe Ile His
            20                  25                  30

Ser Ile Ser Thr Ser Pro Lys Asp Ser Leu Pro Val Pro Ile Tyr Ala
        35                  40                  45

Pro Tyr His Ala Ser His Leu Tyr Ser Met Asp Asp Val Asp Glu Val
    50                  55                  60

Leu Ser Leu Ser Ala Pro Ser Phe Ala Ser Glu Ser Ile Ile Pro Leu
65                  70                  75                  80

Ile Ser Ser Ser Ser Gly Asp Glu Leu Gln Pro Leu Lys Tyr Ala Asp
                85                  90                  95

Leu Leu Arg Cys Cys Val Ser Asp Met Leu Ile Gln Pro Leu Asp Leu
            100                 105                 110

Thr Lys Val Ser Gln Ala Val Ala Gln Leu Leu Glu Val Ser Ser Ser
        115                 120                 125

Thr Arg Ala Ile Ile Lys Pro Ile Ala Thr Ser Val Ser Asn Ser Leu
    130                 135                 140

Val Ser Val Leu Glu Pro Thr Leu Ala Glu Arg Cys Ala Val Asp Asn
145                 150                 155                 160

Ser Met Gly Pro Lys Ala Ser Thr Ser His Ser Ser Ala Glu Thr Gln
                165                 170                 175

Thr Glu Ser Ser Ser Lys Asn Ser Lys Ile Ala Ile Val Ala Met Ser
            180                 185                 190

Gly Arg Phe Pro Asp Ala Ala Asp Leu Ser Glu Phe Trp Asp Leu Leu
        195                 200                 205
```

10

Tyr Glu Gly Arg Asp Val His Arg Gln Ile Pro Glu Asp Arg Phe Asn
210 215 220

Ala Glu Leu His Tyr Asp Ala Thr Gly Arg Arg Lys Asn Thr Ser Lys
225 230 235 240

Val Met Asn Gly Cys Phe Ile Lys Glu Pro Gly Leu Phe Asp Ala Arg
245 250 255

Phe Phe Asn Met Ser Pro Lys Glu Ala Glu Gln Ser Asp Pro Gly Gln
260 265 270

Arg Met Ala Leu Glu Thr Ala Tyr Glu Ala Leu Glu Met Ala Ser Ile
275 280 285

Val Pro Asp Arg Thr Pro Ser Thr Gln Arg Asp Arg Val Gly Val Phe
290 295 300

Tyr Gly Met Thr Ser Asp Asp Trp Arg Glu Val Asn Ser Gly Gln Asn
305 310 315 320

Val Asp Thr Tyr Phe Ile Pro Gly
325

<210> 9
<211> 2022
<212> DNA
<213> Fusarium graminearum

<220>
<221> CDS
<222> (1)..(2022)

<400> 9

```
caa ctt cgc tgg ttt gga ccg agg cca ctt cct atc tca aga acc ggt   48
Gln Leu Arg Trp Phe Gly Pro Arg Pro Leu Pro Ile Ser Arg Thr Gly
 1               5                  10                  15

aag tct ctc aca gat ctt gga agc ttc ctt ctt ttt agt gtt ttt act   96
Lys Ser Leu Thr Asp Leu Gly Ser Phe Leu Leu Phe Ser Val Phe Thr
            20                  25                  30

gac ctt gtt cca ggc aac tgc aac acc ttt gac gat gga gca gac gga   144
Asp Leu Val Pro Gly Asn Cys Asn Thr Phe Asp Asp Gly Ala Asp Gly
        35                  40                  45

tac tgt cga gct gat ggc gtc gga acc atc atc ctc aag cgg ctt gag   192
Tyr Cys Arg Ala Asp Gly Val Gly Thr Ile Ile Leu Lys Arg Leu Glu
    50                  55                  60

gac gcc gaa gct gac aat gac cct att ctc ggt gtc att ctg ggc gct   240
Asp Ala Glu Ala Asp Asn Asp Pro Ile Leu Gly Val Ile Leu Gly Ala
65                  70                  75                  80

tac aca aac cac tca gcc gaa gca gta tcc atc act cga cca cat gcc   288
Tyr Thr Asn His Ser Ala Glu Ala Val Ser Ile Thr Arg Pro His Ala
                85                  90                  95
```

```
gga gct caa gag tac atc ttc tcc aaa ctc ctc cgt gag tcg ggc acc   336
Gly Ala Gln Glu Tyr Ile Phe Ser Lys Leu Leu Arg Glu Ser Gly Thr
          100                 105                 110

gat ccc tac aac gtt agc tac atc gag atg cac ggc aca ggc act caa   384
Asp Pro Tyr Asn Val Ser Tyr Ile Glu Met His Gly Thr Gly Thr Gln
      115                 120                 125

gcc ggc gac gca acc gag atg aca tcc gtc ctc aag acg ttt gct cct   432
Ala Gly Asp Ala Thr Glu Met Thr Ser Val Leu Lys Thr Phe Ala Pro
  130                 135                 140

acc agc ggc ttc ggc ggt cga ttg cct cac caa aac ctt cac ttg ggt   480
Thr Ser Gly Phe Gly Gly Arg Leu Pro His Gln Asn Leu His Leu Gly
145                 150                 155                 160

tca gtc aag gcc aat gtc ggg cac ggt gaa tcc gca tct ggt atc att   528
Ser Val Lys Ala Asn Val Gly His Gly Glu Ser Ala Ser Gly Ile Ile
                165                 170                 175

gct ctg atc aag acg ctg ctt atg atg gag aag aac atg atc ccg ccg   576
Ala Leu Ile Lys Thr Leu Leu Met Met Glu Lys Asn Met Ile Pro Pro
            180                 185                 190

cat tgt ggt atc aag aca aag atc aat cac cat ttt cct acg gat ctc   624
His Cys Gly Ile Lys Thr Lys Ile Asn His His Phe Pro Thr Asp Leu
        195                 200                 205

act cag cgc aat gtc cat atc gcc aaa gtt ccg aca tct tgg aca aga   672
Thr Gln Arg Asn Val His Ile Ala Lys Val Pro Thr Ser Trp Thr Arg
    210                 215                 220

tcg ggt caa gcc aat cca cgc att gct ttc gtc aat aac ttc tct gcc   720
Ser Gly Gln Ala Asn Pro Arg Ile Ala Phe Val Asn Asn Phe Ser Ala
225                 230                 235                 240

gct ggt ggt aac tct gct gtc cta ctg caa gat gct cct cag cca tcg   768
Ala Gly Gly Asn Ser Ala Val Leu Leu Gln Asp Ala Pro Gln Pro Ser
                245                 250                 255

gta gtt tcg gat gtt aca gac cct cgc aca tcc cat gtt gtc act atg   816
Val Val Ser Asp Val Thr Asp Pro Arg Thr Ser His Val Val Thr Met
            260                 265                 270

tcc gct cga tca gca gat tcc ctc agg aag aac ctc gcc aat ctc aag   864
Ser Ala Arg Ser Ala Asp Ser Leu Arg Lys Asn Leu Ala Asn Leu Lys
        275                 280                 285

gag ctt gta gaa ggc caa ggt gac tcg gag gtc ggc ttc ctg agc aag   912
Glu Leu Val Glu Gly Gln Gly Asp Ser Glu Val Gly Phe Leu Ser Lys
    290                 295                 300

ctg tcc tac aca acc acc gcc agg cgc atg cat cat caa ttc cga gct   960
Leu Ser Tyr Thr Thr Thr Ala Arg Arg Met His His Gln Phe Arg Ala
305                 310                 315                 320
```

```
tcg gtc aca gca cag act cgt gaa cag ctg ctg aag ggc ctt gat tcc   1008
Ser Val Thr Ala Gln Thr Arg Glu Gln Leu Leu Lys Gly Leu Asp Ser
                325                 330                 335

gcc att gaa cgc cag gat gtg aag agg atc ccc gcc gcc gcg ccc tct   1056
Ala Ile Glu Arg Gln Asp Val Lys Arg Ile Pro Ala Ala Ala Pro Ser
            340                 345                 350

gtc ggc ttt gtg ttt agc ggc caa ggc gcc caa tac cgt ggt atg ggc   1104
Val Gly Phe Val Phe Ser Gly Gln Gly Ala Gln Tyr Arg Gly Met Gly
        355                 360                 365

aag gag tac ttt aca tct ttc aca gcc ttc cgc tct gag atc atg tct   1152
Lys Glu Tyr Phe Thr Ser Phe Thr Ala Phe Arg Ser Glu Ile Met Ser
    370                 375                 380

tac gac agt atc gcc caa gcc caa ggc ttc ccg tca atc ctc cca ctg   1200
Tyr Asp Ser Ile Ala Gln Ala Gln Gly Phe Pro Ser Ile Leu Pro Leu
385                 390                 395                 400

atc cga gga gag gtg gaa gct gac tcg ttg agt cct gtt gag atc cag   1248
Ile Arg Gly Glu Val Glu Ala Asp Ser Leu Ser Pro Val Glu Ile Gln
                405                 410                 415

ctg ggt ctc act tgc ctg cag atg gca ctg gcc aag cta tgg aag tca   1296
Leu Gly Leu Thr Cys Leu Gln Met Ala Leu Ala Lys Leu Trp Lys Ser
            420                 425                 430

ttc ggt gtt gag cca ggc ttt gtt ctc gga cac agc tta ggc cac tat   1344
Phe Gly Val Glu Pro Gly Phe Val Leu Gly His Ser Leu Gly His Tyr
        435                 440                 445

gct gct tta cac gtc gct ggt gtt ctg tcc gcc aat gat acc att tac   1392
Ala Ala Leu His Val Ala Gly Val Leu Ser Ala Asn Asp Thr Ile Tyr
    450                 455                 460

ctc act ggc atc aga gcg cag ctg ctc gtg gat aag tgc cag gca gga   1440
Leu Thr Gly Ile Arg Ala Gln Leu Leu Val Asp Lys Cys Gln Ala Gly
465                 470                 475                 480

acc cac tca atg ctg gca gtg agg gca tcc tta cta cag atc caa cag   1488
Thr His Ser Met Leu Ala Val Arg Ala Ser Leu Leu Gln Ile Gln Gln
                485                 490                 495

ttc ctc gat gcc aac att cac gag gtt gca tgt gtc aat ggt tca cga   1536
Phe Leu Asp Ala Asn Ile His Glu Val Ala Cys Val Asn Gly Ser Arg
            500                 505                 510

gaa gtc gtc atc agt gga cgc gtt gcc gac att gac cag ctg gtt ggc   1584
Glu Val Val Ile Ser Gly Arg Val Ala Asp Ile Asp Gln Leu Val Gly
        515                 520                 525

ctt ttg tcg gct gac aac atc aag gcg acc cgc gtc aag gtg cca ttt   1632
Leu Leu Ser Ala Asp Asn Ile Lys Ala Thr Arg Val Lys Val Pro Phe
    530                 535                 540
```

```
gcc ttc cac tca gcg cag gtt gac ccc att ctc tcc gac ttg gat aca   1680
Ala Phe His Ser Ala Gln Val Asp Pro Ile Leu Ser Asp Leu Asp Thr
545                 550                 555                 560

gcg gcg tcg cgc gtc acc ttc cac tcc ctc cag att cct gtt ctt tgt   1728
Ala Ala Ser Arg Val Thr Phe His Ser Leu Gln Ile Pro Val Leu Cys
                565                 570                 575

gcc ctt gac agc tct gtc atc agc cct gga aac cat ggt gtc att ggt   1776
Ala Leu Asp Ser Ser Val Ile Ser Pro Gly Asn His Gly Val Ile Gly
            580                 585                 590

ccc ctt cat cta cag cga cat tgt cgt gag aca gtc aac ttt gag ggt   1824
Pro Leu His Leu Gln Arg His Cys Arg Glu Thr Val Asn Phe Glu Gly
        595                 600                 605

gct cta cat gct gcg gag cac gag aag atc atc aac aag aca tca act   1872
Ala Leu His Ala Ala Glu His Glu Lys Ile Ile Asn Lys Thr Ser Thr
    610                 615                 620

cta tgg atc gag att ggt ccc cat gtt gtc tgc tct acc ttc ctc aag   1920
Leu Trp Ile Glu Ile Gly Pro His Val Val Cys Ser Thr Phe Leu Lys
625                 630                 635                 640

tcc agc ctt ggt cca agc aca cct gct atc gca tcg ctt cgc cga aat   1968
Ser Ser Leu Gly Pro Ser Thr Pro Ala Ile Ala Ser Leu Arg Arg Asn
                645                 650                 655

gac gat tgc tgg aag gtg ttg gct gat ggt ttg agc agt ctc tac agc   2016
Asp Asp Cys Trp Lys Val Leu Ala Asp Gly Leu Ser Ser Leu Tyr Ser
            660                 665                 670

agc ggg                                                          2022
Ser Gly
```

<210> 10
<211> 674
<212> PRT
<213> Fusarium graminearum

<400> 10

```
Gln Leu Arg Trp Phe Gly Pro Arg Pro Leu Pro Ile Ser Arg Thr Gly
 1               5                  10                  15

Lys Ser Leu Thr Asp Leu Gly Ser Phe Leu Leu Phe Ser Val Phe Thr
            20                  25                  30

Asp Leu Val Pro Gly Asn Cys Asn Thr Phe Asp Asp Gly Ala Asp Gly
        35                  40                  45

Tyr Cys Arg Ala Asp Gly Val Gly Thr Ile Ile Leu Lys Arg Leu Glu
    50                  55                  60

Asp Ala Glu Ala Asp Asn Asp Pro Ile Leu Gly Val Ile Leu Gly Ala
65                  70                  75                  80
```

Tyr Thr Asn His Ser Ala Glu Ala Val Ser Ile Thr Arg Pro His Ala
85 90 95

Gly Ala Gln Glu Tyr Ile Phe Ser Lys Leu Leu Arg Glu Ser Gly Thr
100 105 110

Asp Pro Tyr Asn Val Ser Tyr Ile Glu Met His Gly Thr Gly Thr Gln
115 120 125

Ala Gly Asp Ala Thr Glu Met Thr Ser Val Leu Lys Thr Phe Ala Pro
130 135 140

Thr Ser Gly Phe Gly Gly Arg Leu Pro His Gln Asn Leu His Leu Gly
145 150 155 160

Ser Val Lys Ala Asn Val Gly His Gly Glu Ser Ala Ser Gly Ile Ile
165 170 175

Ala Leu Ile Lys Thr Leu Leu Met Met Glu Lys Asn Met Ile Pro Pro
180 185 190

His Cys Gly Ile Lys Thr Lys Ile Asn His His Phe Pro Thr Asp Leu
195 200 205

Thr Gln Arg Asn Val His Ile Ala Lys Val Pro Thr Ser Trp Thr Arg
210 215 220

Ser Gly Gln Ala Asn Pro Arg Ile Ala Phe Val Asn Asn Phe Ser Ala
225 230 235 240

Ala Gly Gly Asn Ser Ala Val Leu Leu Gln Asp Ala Pro Gln Pro Ser
245 250 255

Val Val Ser Asp Val Thr Asp Pro Arg Thr Ser His Val Val Thr Met
260 265 270

Ser Ala Arg Ser Ala Asp Ser Leu Arg Lys Asn Leu Ala Asn Leu Lys
275 280 285

Glu Leu Val Glu Gly Gln Gly Asp Ser Glu Val Gly Phe Leu Ser Lys
290 295 300

Leu Ser Tyr Thr Thr Thr Ala Arg Arg Met His His Gln Phe Arg Ala
305 310 315 320

Ser Val Thr Ala Gln Thr Arg Glu Gln Leu Leu Lys Gly Leu Asp Ser
325 330 335

Ala Ile Glu Arg Gln Asp Val Lys Arg Ile Pro Ala Ala Ala Pro Ser
340 345 350

Val Gly Phe Val Phe Ser Gly Gln Gly Ala Gln Tyr Arg Gly Met Gly
355 360 365

Lys Glu Tyr Phe Thr Ser Phe Thr Ala Phe Arg Ser Glu Ile Met Ser
370 375 380

Tyr Asp Ser Ile Ala Gln Ala Gln Gly Phe Pro Ser Ile Leu Pro Leu
385 390 395 400

Ile Arg Gly Glu Val Glu Ala Asp Ser Leu Ser Pro Val Glu Ile Gln
405 410 415

Leu Gly Leu Thr Cys Leu Gln Met Ala Leu Ala Lys Leu Trp Lys Ser
420 425 430

Phe Gly Val Glu Pro Gly Phe Val Leu Gly His Ser Leu Gly His Tyr
435 440 445

Ala Ala Leu His Val Ala Gly Val Leu Ser Ala Asn Asp Thr Ile Tyr
450 455 460

Leu Thr Gly Ile Arg Ala Gln Leu Leu Val Asp Lys Cys Gln Ala Gly
465 470 475 480

Thr His Ser Met Leu Ala Val Arg Ala Ser Leu Leu Gln Ile Gln Gln
485 490 495

Phe Leu Asp Ala Asn Ile His Glu Val Ala Cys Val Asn Gly Ser Arg
500 505 510

Glu Val Val Ile Ser Gly Arg Val Ala Asp Ile Asp Gln Leu Val Gly
515 520 525

Leu Leu Ser Ala Asp Asn Ile Lys Ala Thr Arg Val Lys Val Pro Phe
530 535 540

Ala Phe His Ser Ala Gln Val Asp Pro Ile Leu Ser Asp Leu Asp Thr
545 550 555 560

Ala Ala Ser Arg Val Thr Phe His Ser Leu Gln Ile Pro Val Leu Cys
565 570 575

Ala Leu Asp Ser Ser Val Ile Ser Pro Gly Asn His Gly Val Ile Gly
580 585 590

Pro Leu His Leu Gln Arg His Cys Arg Glu Thr Val Asn Phe Glu Gly
595 600 605

Ala Leu His Ala Ala Glu His Glu Lys Ile Ile Asn Lys Thr Ser Thr
610 615 620

Leu Trp Ile Glu Ile Gly Pro His Val Val Cys Ser Thr Phe Leu Lys
625 630 635 640

Ser Ser Leu Gly Pro Ser Thr Pro Ala Ile Ala Ser Leu Arg Arg Asn
645 650 655

Asp Asp Cys Trp Lys Val Leu Ala Asp Gly Leu Ser Ser Leu Tyr Ser
660 665 670

Ser Gly

<210> 11
<211> 477
<212> DNA
<213> Fusarium graminearum

<220>
<221> CDS
<222> (1)..(477)

<400> 11

```
tgg gag cac aag aat tac tgg ata cag tac aag tac gat tgg tcc ttg   48
Trp Glu His Lys Asn Tyr Trp Ile Gln Tyr Lys Tyr Asp Trp Ser Leu
  1               5                  10                  15

gct aaa ggt gat cct cca att gcc cct aac agc tcg gtt gaa gca gtc   96
Ala Lys Gly Asp Pro Pro Ile Ala Pro Asn Ser Ser Val Glu Ala Val
             20                  25                  30

tca gct tta tca aca ccc tcg gtc cag aag att cta cag gag act tcc  144
Ser Ala Leu Ser Thr Pro Ser Val Gln Lys Ile Leu Gln Glu Thr Ser
         35                  40                  45

ctt gat cag gta ttg act atc gtt gct gag aca gat ctc gcg agc cct  192
Leu Asp Gln Val Leu Thr Ile Val Ala Glu Thr Asp Leu Ala Ser Pro
     50                  55                  60

cta ttg tca gag gtt gcc caa ggt cat cgg gtc aac ggt gtc aaa gtc  240
Leu Leu Ser Glu Val Ala Gln Gly His Arg Val Asn Gly Val Lys Val
 65                  70                  75                  80

tgc aca tct tcc gtg tac gct gat gtt ggc ttg acg ctg ggt aag tac  288
Cys Thr Ser Ser Val Tyr Ala Asp Val Gly Leu Thr Leu Gly Lys Tyr
                 85                  90                  95

att ttg gac aac tac cgc acc gac tta gag ggt tat gcg gtc gat gtt  336
Ile Leu Asp Asn Tyr Arg Thr Asp Leu Glu Gly Tyr Ala Val Asp Val
            100                 105                 110

cac ggt att gag gtc cac aag cca ctt ctt ctc aaa gaa gac atg aac  384
His Gly Ile Glu Val His Lys Pro Leu Leu Leu Lys Glu Asp Met Asn
        115                 120                 125

gga acg ccc cag gct aca ccg ttc cgc atc gaa gtg cga tac cca atc  432
Gly Thr Pro Gln Ala Thr Pro Phe Arg Ile Glu Val Arg Tyr Pro Ile
    130                 135                 140

cag agc acc acg gcg ctg atg agc atc tcc acc act ggc ccc aac      477
Gln Ser Thr Thr Ala Leu Met Ser Ile Ser Thr Thr Gly Pro Asn
145                 150                 155
```

<210> 12
<211> 159
<212> PRT
<213> Fusarium graminearum

<400> 12

```
Trp Glu His Lys Asn Tyr Trp Ile Gln Tyr Lys Tyr Asp Trp Ser Leu
 1               5                  10                  15

Ala Lys Gly Asp Pro Pro Ile Ala Pro Asn Ser Ser Val Glu Ala Val
            20                  25                  30

Ser Ala Leu Ser Thr Pro Ser Val Gln Lys Ile Leu Gln Glu Thr Ser
        35                  40                  45

Leu Asp Gln Val Leu Thr Ile Val Ala Glu Thr Asp Leu Ala Ser Pro
    50                  55                  60

Leu Leu Ser Glu Val Ala Gln Gly His Arg Val Asn Gly Val Lys Val
65                  70                  75                  80

Cys Thr Ser Ser Val Tyr Ala Asp Val Gly Leu Thr Leu Gly Lys Tyr
                85                  90                  95

Ile Leu Asp Asn Tyr Arg Thr Asp Leu Glu Gly Tyr Ala Val Asp Val
            100                 105                 110

His Gly Ile Glu Val His Lys Pro Leu Leu Leu Lys Glu Asp Met Asn
        115                 120                 125

Gly Thr Pro Gln Ala Thr Pro Phe Arg Ile Glu Val Arg Tyr Pro Ile
    130                 135                 140

Gln Ser Thr Thr Ala Leu Met Ser Ile Ser Thr Thr Gly Pro Asn
145                 150                 155
```

<210> 13
<211> 510
<212> DNA
<213> Fusarium graminearum

<220>
<221> CDS
<222> (1)..(510)

<400> 13

```
tgc gaa ctt cga ctc gag cat ccg tcg caa tgg gaa gcg gag tgg gat   48
Cys Glu Leu Arg Leu Glu His Pro Ser Gln Trp Glu Ala Glu Trp Asp
 1               5                  10                  15

cgc caa gcc tac ctc atc aat cgc agc gtc aac tgc ctt ctg cag cga   96
Arg Gln Ala Tyr Leu Ile Asn Arg Ser Val Asn Cys Leu Leu Gln Arg
            20                  25                  30

tca gca caa ggt ttg gac agc atg ttg gca acc gga atg gtc tac aag   144
Ser Ala Gln Gly Leu Asp Ser Met Leu Ala Thr Gly Met Val Tyr Lys
        35                  40                  45

gtc ttc tcc tcc ctc gtc gac tat gcc gat ggc tac aag ggt ctg cag   192
Val Phe Ser Ser Leu Val Asp Tyr Ala Asp Gly Tyr Lys Gly Leu Gln
    50                  55                  60
```

```
gag gtt gtc ttg cac agc caa gag ctc gag ggc aca gca aaa gtg cgc   240
Glu Val Val Leu His Ser Gln Glu Leu Glu Gly Thr Ala Lys Val Arg
 65                  70                  75                  80

ttc caa act ccc tcg gga ggt ttc gtc tgc aat ccc atg tgg att gac   288
Phe Gln Thr Pro Ser Gly Gly Phe Val Cys Asn Pro Met Trp Ile Asp
                85                  90                  95

agc tgt ggt cag acg acc ggc ttc atg atg aac tgt cat cag act acg   336
Ser Cys Gly Gln Thr Thr Gly Phe Met Met Asn Cys His Gln Thr Thr
            100                 105                 110

ccc aat gac tac gtc tac gtc aat cat ggc tgg aag tcg atg aga ttg   384
Pro Asn Asp Tyr Val Tyr Val Asn His Gly Trp Lys Ser Met Arg Leu
        115                 120                 125

gcc aag gcg ttc cgt gaa gat ggt acc tat cgg act tat atc cgg atg   432
Ala Lys Ala Phe Arg Glu Asp Gly Thr Tyr Arg Thr Tyr Ile Arg Met
    130                 135                 140

agg ccc att gat agc acc aag ttc gct ggt gac ttg tac att ctt gat   480
Arg Pro Ile Asp Ser Thr Lys Phe Ala Gly Asp Leu Tyr Ile Leu Asp
145                 150                 155                 160

gag gat gac act gtg gtt ggt gtt tat gga                           510
Glu Asp Asp Thr Val Val Gly Val Tyr Gly
                165                 170
```

<210> 14
<211> 170
<212> PRT
<213> Fusarium graminearum

<400> 14

```
Cys Glu Leu Arg Leu Glu His Pro Ser Gln Trp Glu Ala Glu Trp Asp
  1               5                  10                  15

Arg Gln Ala Tyr Leu Ile Asn Arg Ser Val Asn Cys Leu Leu Gln Arg
             20                  25                  30

Ser Ala Gln Gly Leu Asp Ser Met Leu Ala Thr Gly Met Val Tyr Lys
         35                  40                  45

Val Phe Ser Ser Leu Val Asp Tyr Ala Asp Gly Tyr Lys Gly Leu Gln
     50                  55                  60

Glu Val Val Leu His Ser Gln Glu Leu Glu Gly Thr Ala Lys Val Arg
 65                  70                  75                  80

Phe Gln Thr Pro Ser Gly Gly Phe Val Cys Asn Pro Met Trp Ile Asp
                85                  90                  95

Ser Cys Gly Gln Thr Thr Gly Phe Met Met Asn Cys His Gln Thr Thr
            100                 105                 110
```

```
Pro Asn Asp Tyr Val Tyr Val Asn His Gly Trp Lys Ser Met Arg Leu
        115                 120                 125

Ala Lys Ala Phe Arg Glu Asp Gly Thr Tyr Arg Thr Tyr Ile Arg Met
    130                 135                 140

Arg Pro Ile Asp Ser Thr Lys Phe Ala Gly Asp Leu Tyr Ile Leu Asp
145                 150                 155                 160

Glu Asp Asp Thr Val Val Gly Val Tyr Gly
                165                 170
```

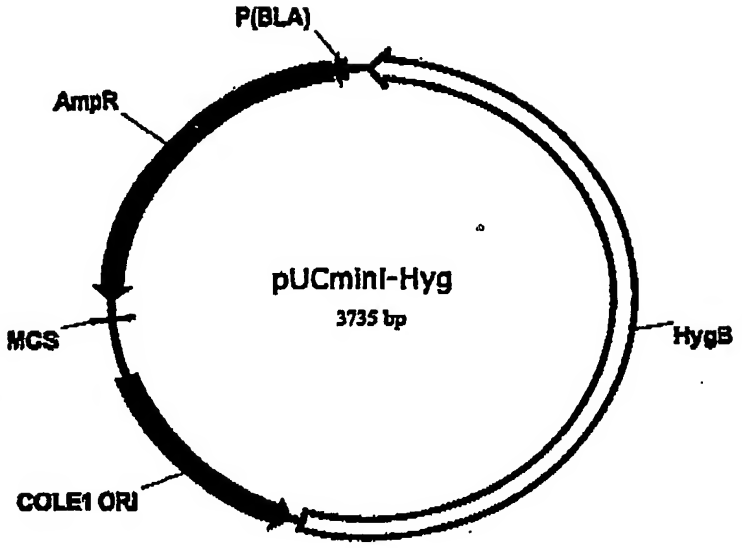

Figure 1

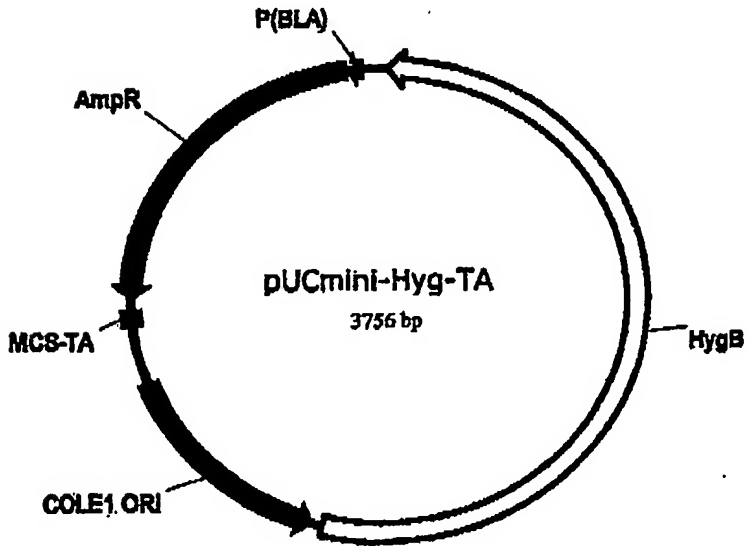

Figure 2